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# Genetic Studies of Wildlife

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# GENETIC STUDIES OF WILDLIFE

by

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GENETIC STUDIES OF WILDLIFE

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Genetic techniques are being more frequently used to understand the biology and management of wildlife species. The wild turkey is one species of genetic interest because the correct identification of individuals to the subspecies level is difficult using traditional methods. Currently phenotypic differences in plumage, especially the upper tail coverts, are used to assign individuals to subspecies. To hunters wanting to complete a “grand slam,” identification of birds’ subspecies is important. This study focuses on the five extant subspecies: Eastern (*M. g. silvestris*), Osceola (*M. g. osceola*), Rio Grande (M. *g. intermedia*), Merriam’s (*M. g. merriami*), and Gould’s (*M. g. mexicana*). I aimed to determine if molecular genetic data provide support for currently recognized subspecies. I also attempted to determine if quantitative measurements of coloration of the upper tail coverts is geographically discrete and consistent with historical subspecies boundaries. I used primer sets for 11 single nucleotide polymorphisms thought to be diagnostic at the subspecies level and sequenced DNA of tissue samples from 81 birds to determine whether they were pure examples of a subspecies or hybrids. To measure plumage coloration, I used a spectrophotometer to obtain quantitative measurements of upper tail coverts from individuals obtained in 21 states and all subspecies. Genetic analyses suggested that most wild turkeys in Nebraska represent a mixture of many subspecies. Morphological analyses indicated that there are not five distinct spectral ranges that

correspond with accepted subspecies, but most likely two that roughly divide turkeys from east to west. These analyses plus comparison of mitochondrial genomes suggests that the genetic landscape of wild turkey is basically divided into eastern and western groups. To explore the use of molecular phylogenetics in wildlife genetics I also did a study on the evolution of Transmissible Spongiform Encephalopathies across 102 species of mammals. Phylogenetic hypotheses for the prion protein gene, thought to be responsible for transmissible spongiform encephalopathies, and a species tree inferred from 20 unlinked nuclear genes, were compared, finding highly congruent topologies. Mapping the presence/absence of TSEs on the species tree, TSEs occur non-randomly and have arisen independently and recently in different mammalian groups. This suggests that the evolution of TSEs develops in groups of species irrespective of PRNP genotype.

## AUTHOR’S ACKOWLEDGMENTS

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# TABLE OF CONTENTS

# LISTS OF MULTIMEDIA OBJECTS

**LIST OF FIGURES**

**LIST OF TABLES**

# CHAPTER 1: WILD TURKEY SUBSPECIES

## 1.1 ABSTRACT

Genetic techniques are being more frequently used to understand the biology and management of wildlife species. The wild turkey is one species of genetic interest because the correct identification of individuals to the subspecies level is difficult using traditional methods. Currently phenotypic differences in plumage, especially the upper tail coverts, are used to assign individuals to subspecies. Whether the subspecies are genetically distinct is still unclear. I aimed to determine if molecular genetic data provide support for currently recognized subspecies. Using primer sets for 11 single nucleotide polymorphisms thought to be diagnostic at the subspecies level and tissue samples from 81 birds to determine whether they were pure examples of a subspecies or hybrids. Using the population analysis tool STRUCTURE, two distinct genetic groups were found with no pure individuals. Genetic analyses suggested that most wild turkeys in Nebraska represent a mixture of many subspecies. These analyses plus comparison of mitochondrial genomes suggests that the genetic landscape of wild turkey is basically divided into eastern and western groups.

## 1.2 INTRODUCTION

The wild turkey (*Meleagris gallopavo*) is a large Galliform bird endemic to North America. In the past, due to overharvest and habitat degradation, the wild turkey was extirpated from its historic range (Appendix A; Fig 1.1). Due to restoration efforts, the turkey has made a remarkable recovery. Today, the wild turkey can be found throughout North America, far beyond its historic range (Appendix A; Fig 1.2). There are currently six subspecies recognized by taxonomists, five of which are extant, and one from southern Mexico (*M. g. gallopavo*) thought to be extinct (Hughes & Lee 2015; McRoberts et al. 2014). The five extant subspecies are: Eastern (*M. g. silvestris*), Osceola (*M. g. osceola*), Rio Grande (*M. g. intermedia*), Merriam (*M. g. merriami*), and Gould's (*M. g. mexicana*). Each of the subspecies are distinguished by their historic geographic ranges and morphological features. The most common morphological features used to identify subspecies are coloration of the upper tail coverts, coloration of the band at the end of the tail, black and white barring of the primary wing feathers, and body size (Hughes and Lee 2015).

The color of the tip of the upper tail covert grades between subspecies, and hence, only at the extremes of the coloration continuum does it serve to distinguish between the subspecies. The Osceola wild turkey has dark brown tips with the color gradually getting lighter through Eastern, and Rio, with Gould's and Merriam's having pure white tips (Kennamer et al. 1992; McRoberts et al. 2014). Although Gould's and Merriam's both have pure white upper tail coverts, the Gould's typically have a buffy body color compared to the darker Merriam's.

The identification of wild turkey subspecies is of interest to wildlife managers and hunters for a variety of reasons. The ability to identify the subspecies to which an individual belongs will assist management efforts to reintroduce subspecies found historically in their areas. Possible re-establishment of the historic subspecies can result in the establishment of individuals carrying the genetic composition of populations that originally lived in that area to mitigate changes to the genetic diversity of turkeys. In contrast, promoting hybrid vigor may provide benefits to the population, while diluting genetic distinctness between subspecies. Subspecies identification is also important for hunters because many wild turkey hunters wish to accomplish a "Grand Slam," which involves harvesting four subspecies (Eastern, Osceola, Rio, and Merriam), a "Royal Slam" with the addition of the Gould's turkey, and a "World Slam" with the addition of the Ocellated turkey (*Meleagris ocellata*), which only occurs in Mexico. Correct identification of individuals of each subspecies is made difficult by the presence of natural and man-made hybrids, which is of concern to hunters attempting one of these slams.

Wild turkeys were extirpated from Nebraska and required translocations to re-establish the population. Because it has been suggested that Nebraska includes three subspecies, I chose to analyze the current genetic makeup of Nebraska wild turkeys. Lusk (in Wagner 2018) noted that the majority of wild turkey re-introductions during in the 1990's in Nebraska were of intentionally hybridized turkeys (Merriam's crossed with game farm Eastern's). Though, Lusk adds, there were releases of putative pure Merriam's, Rio Grande's and a few Eastern's. Lusk (personal, communication, 2018) suggests that given current knowledge, Nebraska's turkeys are hybrids, which is reflected in the map shown by the National Wild Turkey Federation (https://www.nwtf.org/hunt/wild-turkey-basics/habitat). Some outfitters in Nebraska advertise to assist their clients in harvesting examples of pure Merriam's, Rio or Eastern turkeys, and my study will clarify whether this is the possible (J. Lusk, personal communication).

Research has attempted to identify genetic markers for each subspecies of turkey, to infer the relationships between the subspecies and ascertain how past reintroduction efforts have influenced the genetic composition of local populations. Mock et al. (2002)

characterized the genetic diversity of wild turkey populations in each of the 5 known subspecies' ranges using a combination of the mitochondrial control region and nuclear DNA-based markers. Their phylogenetic hypothesis suggested that Gould's turkeys were the most genetically divergent from the other taxa. Results also indicated that Gould's had the least diversity with respect to mitochondrial diversity, though the remaining subspecies had similar levels of genetic variability. They found support for the existence of the other subspecies. However, Latch (2005) found that although there are distinct differences in the cytochrome *b* gene sequences between eastern and western subspecies, no definitive geographic, or subspecies-specific structuring had accrued due to the slow evolutionary rate of the gene (Latch et al. 2006) or recent and incomplete geographic isolation. The level of gene flow between turkey populations in close proximity has been suspected to be low (Szalanski et al. 2000). However, 19 years following an introduction event, microsatellite data indicated that the genetic integrity of the introduced population of Merriam's turkeys in the Davis Mountains Preserve has been eroded by both immigration from and hybridization with nearby Rio Grande populations (Latch et al. 2006).

Despite some indications of the relationships between subspecies, no studies have determined whether all five subspecies are separated by diagnostic genetic or morphological differences at subspecies boundaries, especially in cases where zones of integration have been noted such as that between Eastern and Osceola subspecies (Aldrich 1967). Furthermore, it is unknown if ranges determined by morphological characteristics (Szalanski et al. 2000) are congruent with genetic differences. As noted by Stangel et al. (1992), "boundaries of subspecies are subjective". This is typical of many avian subspecies (Zink 2004) because there is no set standard for naming subspecies (Cronin 1993). Like many scientific classifications this can change over time with technological advancements, including genetic techniques. An obvious test of the validity of morphologically determining subspecies is to discover whether each subspecies has diagnostic genetic differences. This study aims to use diagnostic single nucleotide polymorphisms (SNPs) derived from the nuclear genome, and entire mitochondrial genomes, to resolve whether there are five distinct geographical groupings of wild turkey.

## 1.3 METHODS

### 1.3.1 DETERMINING SNP LOCATIONS

To identify diagnostic SNPs, a low coverage whole genome analysis was performed using 16 museum specimens that were determined to be putatively ‘pure’ individuals of each subspecies and the ocellated species. These specimens were collected after 1980 from Massachusetts, New Jersey, New York, Florida, Texas, New Mexico and Arizona, hence they do not necessarily represent individuals from untainted sources and geographic localities.  Using 8554 SNPs that received 5X coverage scores (Vázquez-Miranda, pers. comm.), a phylogenetic analysis was performed to determine if the individuals grouped according to presumptive subspecies membership. The tree (Fig. 1.15) supported each subspecies, with the exception of an individual identified as a Merriam’s (MTX0a,b) that is most likely a Rio Grande.  After finding that the subspecies were supported by the phylogenetic analysis (Fig 1.15), 16 diagnostic SNPs were identified (4 for Eastern, 2 for Gould’s, 4 for Merriam’s, 2 for Osceola, and 4 for Rio Grande). Primers were created for each SNP to use on all samples of turkeys from Nebraska and elsewhere (excluding those used to develop the primers). Due to some primers showing double bands in the PCR product resulting in large amounts of missing data only 11 diagnostic SNPs were used for analyses, with at least two from each subspecies (Appendix A, Table 1.1).

### 1.3.2 GENETIC DATA PROCEDURES

In Spring 2016, I obtained 58 wild turkey tissue samples gathered by Nebraska Game and Parks Commission (NGPC) biologists from hunters in Nebraska as well as 22 samples from hunters in Wisconsin, Texas, California, Minnesota, Florida, Missouri, and North Carolina. I obtained 11 domestic turkey tissue samples in 2018 from a local grocery store in Lincoln, NE resulting in 91 tissue samples. For analyses I excluded individuals that did not have diagnostic SNP data for every subspecies, resulting in the exclusion of 10 individuals, which include our two individuals from Florida. 81 samples were therefore used for analyses.

Tissue samples were digested overnight at 58°F in 0.5ml of a protease K solution (10μL of protease K standard TBE buffer). DNA extraction was performed using Phenol-Chloroform methods as described by Miller et al. (1988). DNA concentration was measured and PCR protocols were as follows: 95°C for 3 minutes followed by 30 cycles of 95°C for 30 seconds, an annealing temperature ranging from 57-67°C (varied by primer and sample) for 30 seconds, and 72°C for 10 minutes. 2μL of PCR product were run in a 1% agarose electrophoresis gel and acceptable samples were sent to the company Genewiz (733 Concord Ave. Cambridge, MA 02138) for sequencing. Results were downloaded as *.abi files.

### 1.3.3 STRUCTURE MODEL ANALYSIS

Sequences were processed, aligned to a reference sequence, and edited in the program Geneious Prime 2020.0.5. A data matrix containing the SNPs at diagnostic base positions for the 81 individuals was inputted into the program STRUCTURE. STRUCTURE is a population analysis tool for estimating the number of distinct genetic clusters in a data set. It uses a Bayesian-based method to assign individuals to a population while minimizing Hardy-Weinberg disequilibrium. I set the program to evaluate several cluster (K) values from 1 and 8. The inclusion of clusters greater than 5 allow for the potential of finding all 5 subspecies as well as some subspecies potentially being divided into multiple clusters. Each cluster value was analyzed 20 times with a 5,000 burn-in and 50,000 replicates.

The results obtained were entered into STRUCTURE HARVESTER (Earl and von Holdt 2012). This program outputs a plot of mean likelihood values per cluster that estimates which cluster value has the best fit. STRUCTURE HARVESTER also executes the Evanno method (Evanno et al. 2005), which estimates the number of clusters that best fit the data set (Earl and vonHoldt 2012). The Evanno method uses an ad hoc statistic called change of K, which shows the change of log probability to the number of clusters assigned for analysis (Evanno et al. 2005). The program CLUMPP (Jakobsson and Rosenberg 2007) was used to align the clusters across the 20 runs for improved visualization and the resulting files were input into R for plotting. Domestic samples were excluded due to their unknown source of origin, other than a local grocery store. I produced maps showing the geographic location of the 72 wild turkeys and their genetic subspecies ancestry. The goal was to determine if the most highly supported number of clusters matches the number of subspecies previously hypothesized to exist in Nebraska by visually determining if they overlapped with accepted subspecies ranges.

### 1.3.4 SUBSPECIES SNP PROPORTION PER INDIVIDUAL ANALYSIS

I calculated the proportion of diagnostic alleles that were present within an individual. This method was included to provide a comparison with the STRUCTURE results. I counted the number of diagnostic alleles for each subspecies, which was then divided by the total number of diagnostic alleles found within the individual to find the percentage of a subspecies within a single turkey. For example, if a turkey had two diagnostic alleles (out of the 6 possible) for the Eastern subspecies, one (out of 4 possible) for Merriam's, and zero for the rest I scored that individual as 66% Eastern and 33% Merriam's.  An individual turkey would be considered "pure" if it was homozygous for all of the diagnostic SNPs for a particular subspecies, whereas those that had diagnostic SNPs from more than one subspecies were considered of mixed ancestry.

I also examined these data from the viewpoint of two broad East and West genetic groups of turkeys, which was found in a study of mitogenomes (Fig 1.3) (Vázquez-Miranda et al. in prep.). To compare eastern and western groups, the diagnostic allele counts of Eastern, Osceola, and Rio Grande were combined whereas Gould's and Merriam's were combined to create the West group. Geographic and bar plot representations were created for each scenario.

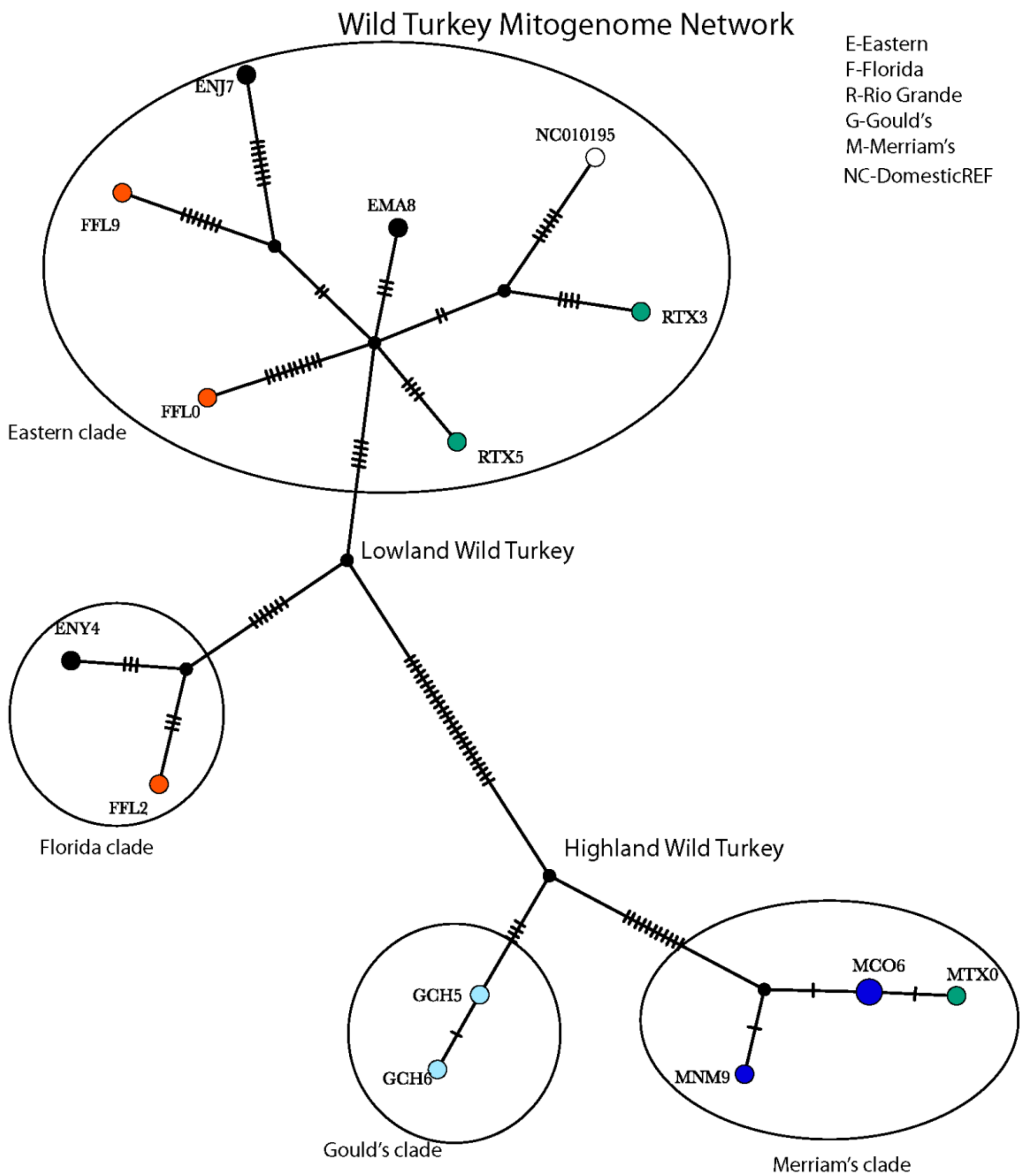


**Fig 1.3 Wild Turkey Mitogenome Network.** Network created from entire mitogenome of putatively pure individuals used to create diagnostic SNPs. Distinct clades are circled, with each mark on branches representing a single mutation. Two broad clades labeled as Highland Wild Turkey and Lowland Wild Turkey which are separated by 25 mutations. Individual nodes are colored by subspecies designation, first letter of labels designate subspecies assignment followed by state abbreviation of the locality.

## 1.4 RESULTS

### 1.4.1 STRUCTURE RESULTS

My results suggested two genetic clusters for the 81 individuals (Figs 1.4, 1.5). No individuals were found that included only alleles of one cluster, indicating the absence of genetically (or subspecifically) pure turkeys. Of the 51 (63%) individuals in Cluster 2, 72.6% (n = 37) were from Nebraska and 17.7% (n = 9) were domestic individuals (represent all the domestics in our data). Individuals from states other than Nebraska made up 7.8% (n = 4) of this group. Among the 30 (37%) individuals in Cluster 1, 60% (n = 18) were from Nebraska and 37% (n = 11) were other states. Nebraska represented a potential hybrid zone (Fig 1.6). Of the fifteen individuals that were from outside of Nebraska, 73% were comprised mostly of Cluster 1 (n = 11). All individuals from Texas (n = 3) and Minnesota (n = 2) were in Cluster 1. Some of the individuals from Missouri (n = 1), California (n = 1), North Carolina (n = 1), and Wisconsin (n = 3) were in Cluster 1. Four individuals that were comprised of Cluster 2 were from Missouri (n = 1), California (n = 1), North Carolina (n = 1), and Wisconsin (n =1).

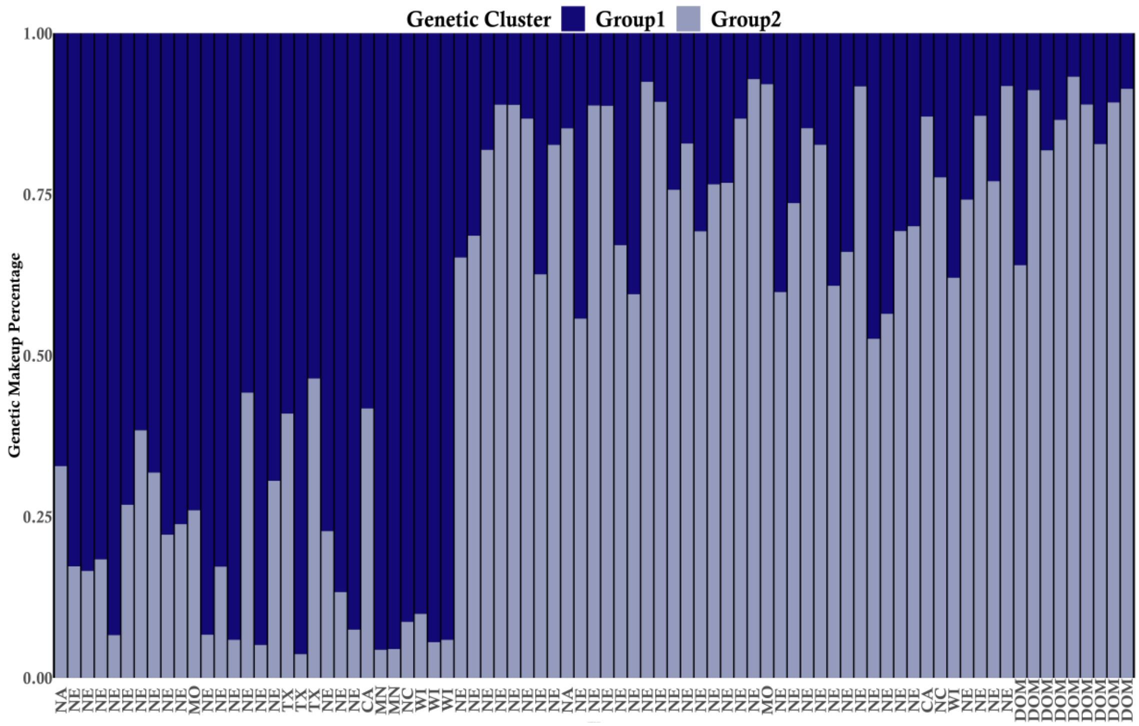


**Fig 1.4 STRUCTURE Plot of All Samples.** STRUCTURE plot for 81 individuals: 55 from NE, 4 from WI, 3 from TX, 2individuals each from MO, CA, MN, NC, 9 domestics obtained from a grocery store, and 2 from unknown locations (NA). Each color represents a distinctive genetic cluster (Blue = Cluster 1 and Gray = Cluster 2) and each bar represents a single individual. The amount of each color in a bar shows how much an individual is made up of that cluster. If five subspecies were distinct, there should be five and not two groups.

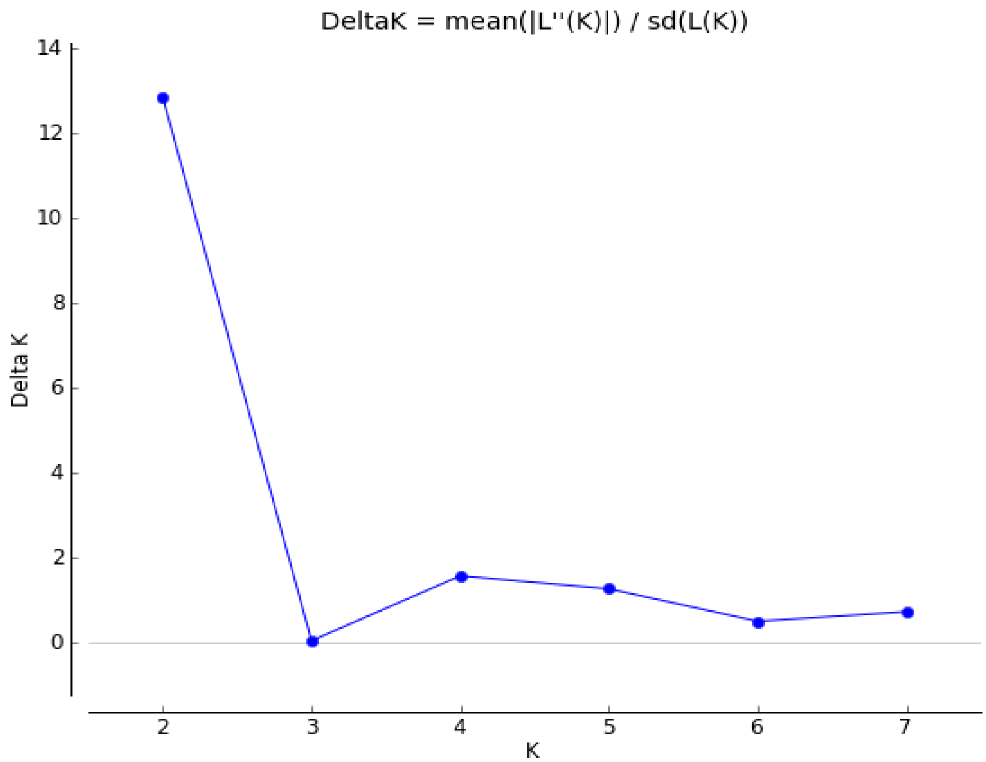


**Fig 1.5 Plot of Delta K values.** STRUCTURE delta K plot for finding best fit K for data using the Evanno (2005) method.

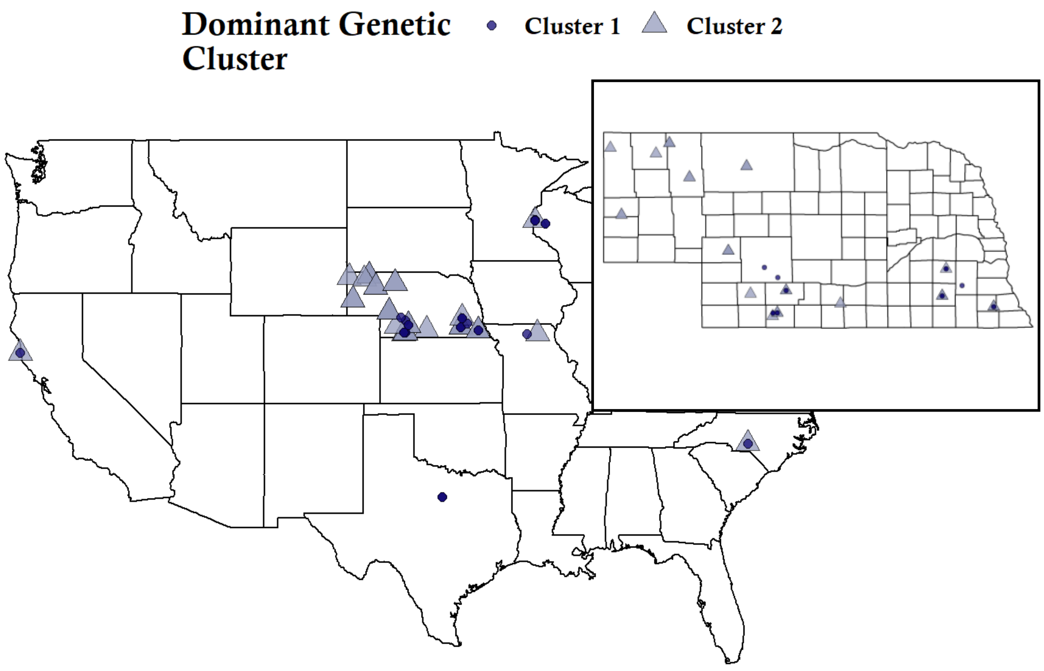


**Fig 1.6 Distribution of genetic clusters**. Map of all individuals with localities marked by which STRUCTURE cluster an individual is made up of the most. Domestics not included.

Nebraska individuals as primarily genetically comprised of Cluster 2 (Fig 1.7). From a geographic standpoint, individuals made up mostly of Cluster 1 are in the Southeast and Southwest portions of the state (Fig. 1.8). In comparison, individuals comprised mostly of Cluster 2 are widespread throughout the state.

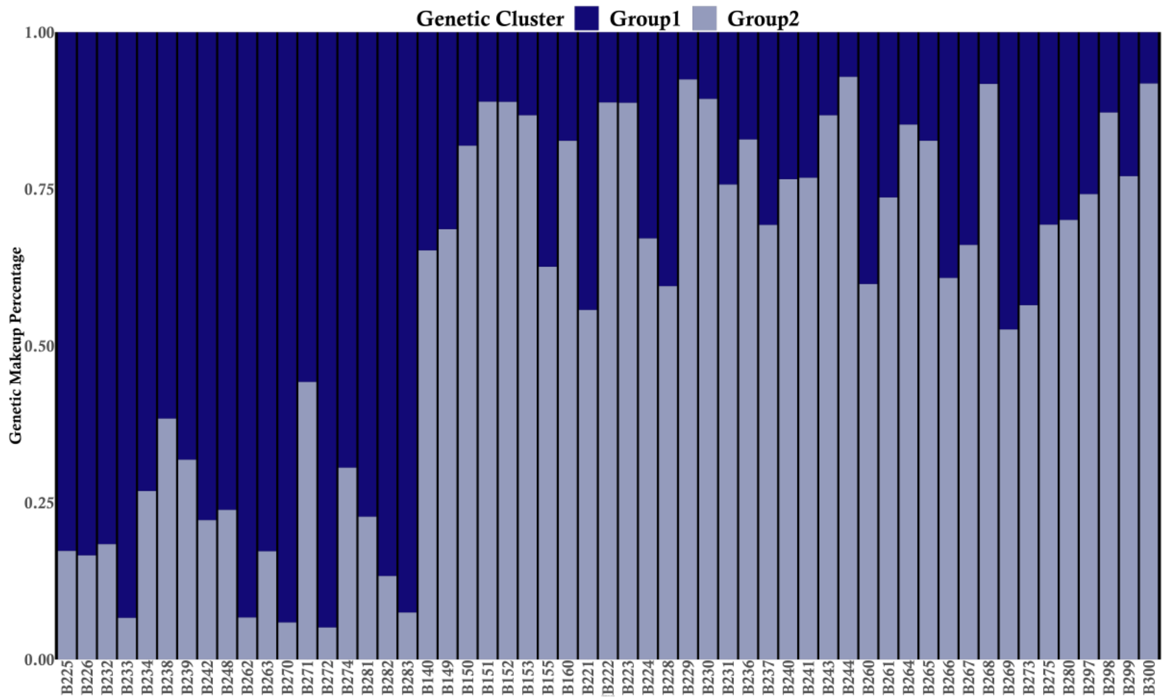


**Fig 1.7 STRUCTURE Plot of Nebraska Samples.** STRUCTURE plot for 55 individuals obtained from Nebraska. Each color represents a distinctive genetic cluster (Blue = Cluster 1 and Gray = Cluster 2) and each bar is 1 individual. The amount of each color in a bar shows the proportion of an individual of each genetic cluster.

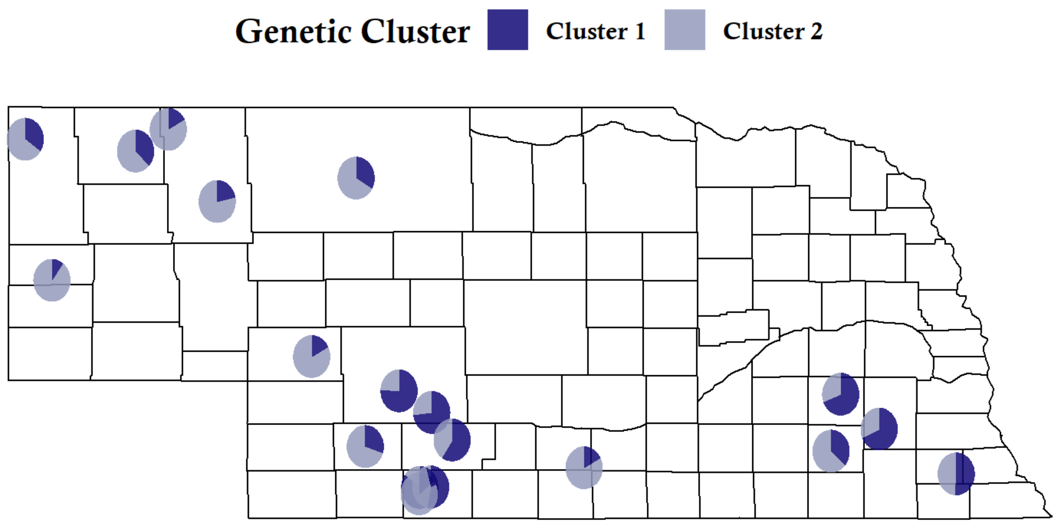


**Fig 1.8 Distribution of Genetic clusters in Nebraska**. Map of NE individuals as pies filled according to proportion of each STRUCTURE group. Domestics not included.

### 1.4.2 SNP RESULTS AS A FUNCTION OF SUBSPECIES

The STRUCTURE results suggested the existence of two and not five genetic groupings. When I conducted the analysis to focus on the 11 subspecies-diagnostic SNPs, including only individuals with data for at least one diagnostic SNP per subspecies (which excluded the two Florida samples), individuals were a mixture of all five subspecies (Fig 1.9). Surprisingly, even SNPs from Osceola and Gould's occurred in individuals far from the current ranges of these subspecies. The most abundant individual makeup for the SNPs were individuals that only had the presence of the Eastern subspecies, which made up 14.10% of the data (Table 1.2). The next most abundant were individuals with both Eastern and Rio Grande SNPs that made up 10.26% of the dataset (Table 1.2). Surprisingly, individuals with both Eastern and Gould's SNPs were the third most prevalent haplotype at 7.69%. Overall, Eastern SNPs were the most detected SNP out of all the subspecies and were found in 60.26% of individuals.

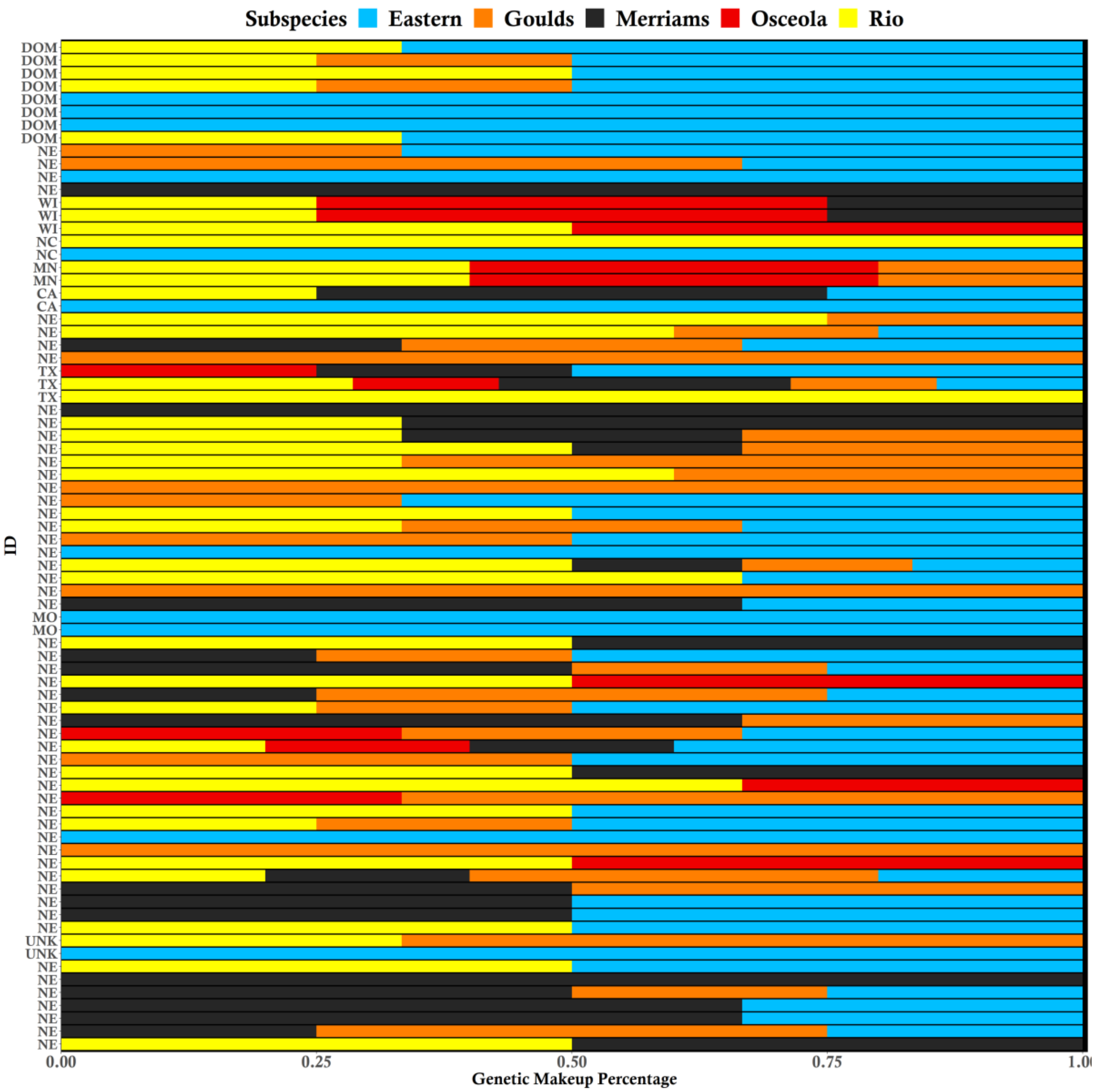


**Fig 1.9 Barplot showing subspecies compositions of individual wild turkeys** including 54 from NE, 3 from WI, 3 from TX, 2 individuals each from MO, CA, MN, NC, 8 domestics obtained from the grocery store, and 2 from unknown locations (UNK). Each color represents a subspecies and each bar represents an individual.

**Table 1.2 Percentage subspecies composition for all individuals.** Subspecies combinations for all 81 individuals. Subspecies names are in the first row, with possible combinations to that subspecies in the first column. Percentages for specific combinations are entered where the first row and column meet. Values in blue highlight the largest percentages.

| | Eastern | Gould’s | Merriam’s | Osceola | Rio |
|---|---|---|---|---|---|
| Eastern | **14.10** | - | - | - | - |
| Gould’s | 6.41 | 5.13 | - | - | - |
| Merriam’s | 6.41 | 3.85 | 3.85 | - | - |
| Osceola | 0.00 | 1.28 | 0.00 | 0.00 | - |
| Rio | **10.26** | 5.13 | 5.13 | 5.13 | 2.56 |
| Eastern + Gould’s | - | - | 6.41 | 1.28 | **7.69** |
| Eastern + Merriam’s | - | - | - | 1.28 | 1.28 |
| Gould's + Merriam's | - | - | - | 0.00 | 2.56 |
| Gould's + Osceola | - | - | - | - | 2.56 |
| Merriam’s + Osceola | - | - | - | - | 2.56 |
| Eastern + Gould's + Merriam's | - | - | - | 0.00 | 2.56 |
| Eastern + Merriam's + Osceola | - | - | - | - | 1.28 |
| Gould's + Merriam's + Osceola | - | - | - | - | 0.00 |
| Eastern + Gould's + Merriam's + Osceola | - | - | - | - | 1.28 |

In Nebraska individuals with Eastern, Merriam's, and Gould's SNPs were the most prevalent in the state (Table 1.3). Almost 60% of individuals possessed an Eastern SNP. The combination of Eastern + Gould's, Eastern + Merriam's, and Eastern + Rio Grande was found in 9.26% of individuals (Table 1.3). Some individuals possessed SNPs from only one subspecies, including three individuals with only Eastern SNPs, four with only Gould's, and three with only Merriam's (Appendix A. Fig 1.10). There were also individuals that had Osceola SNPs. Geographically, it appears that the presence of each subspecies SNP is random, although Rio Grande SNPs are more prevalent in the west (Fig 1.11).

**Table 1.3 Nebraska Subspecies Makeup Table**. Subspecies combinations for 54 individuals from Nebraska. Subspecies names are in the first row, with possible combinations to that subspecies in the first column. Percentages for specific combinations are entered where the first row and column meet. Values in blue highlight the largest percentages.

| | Eastern | Gould’s | Merriam’s | Osceola | Rio |
|---|---|---|---|---|---|
| Eastern | 5.56 | - | - | - | - |
| Gould’s | **9.26** | 7.41 | - | - | - |
| Merriam’s | **9.26** | 3.70 | 5.56 | - | - |
| Osceola | 0.00 | 1.85 | 0.00 | 0.00 | - |
| Rio | **9.26** | 5.56 | 7.41 | 5.56 | 0.00 |
| Eastern + Gould’s | - | - | **11.11** | 1.85 | 7.41 |
| Gould's + Merriam's | - | - | - | 0.00 | 3.70 |
| Merriam’s + Osceola | - | - | - | - | 3.70 |
| Eastern + Gould's + Osceola | - | - | - | - | 1.85 |

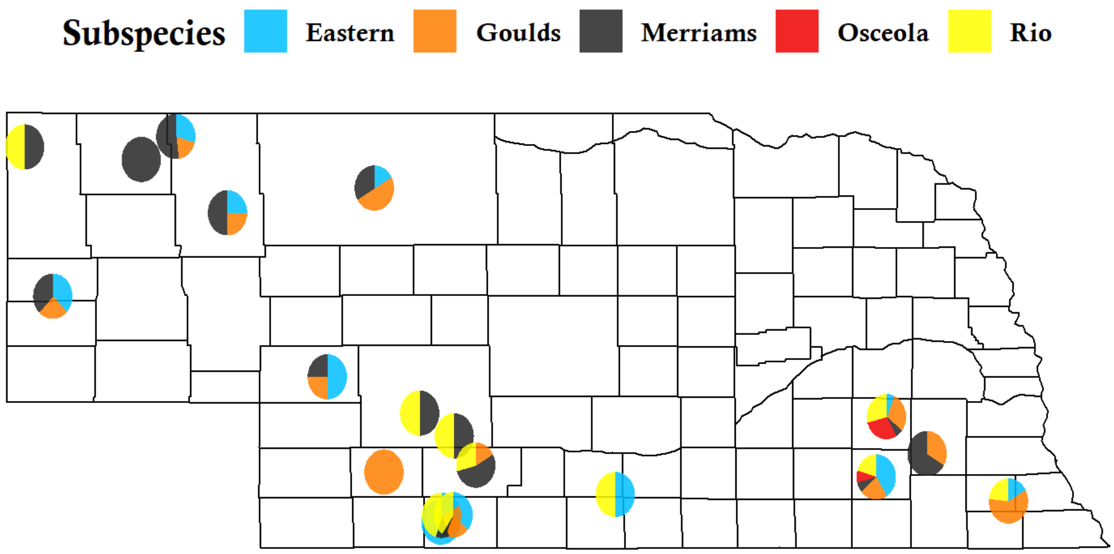


**Fig 1.11 Subspecies representation in Nebraska**. Map of NE with individuals as pies filled according to total proportion of each subspecies diagnostic SNPs within individuals. *Note that pies showing all 5 subspecies are many different points overlaid on top one another, there are no individuals in NE that are made up of all 5 subspecies. Domestics not included.

### 1.4.3 DIAGNOSTIC SNP RESULTS AS EAST/WEST PROPORTIONS

Inspecting the analysis of entire mitochondrial genomes, derived from the same specimens used to develop diagnostic SNPs, separated Gould’s and Merriam’s from individuals representing Eastern, Rio Grande, and Osceola (Fig. 1.3) (Vázquez-Miranda et al. in prep.). Reanalyzing SNPs by combining subspecies into these two groups, results (Fig 1.12) also show East and West groups geographically, with Nebraska in the middle as a hybrid zone (Fig 1.13).

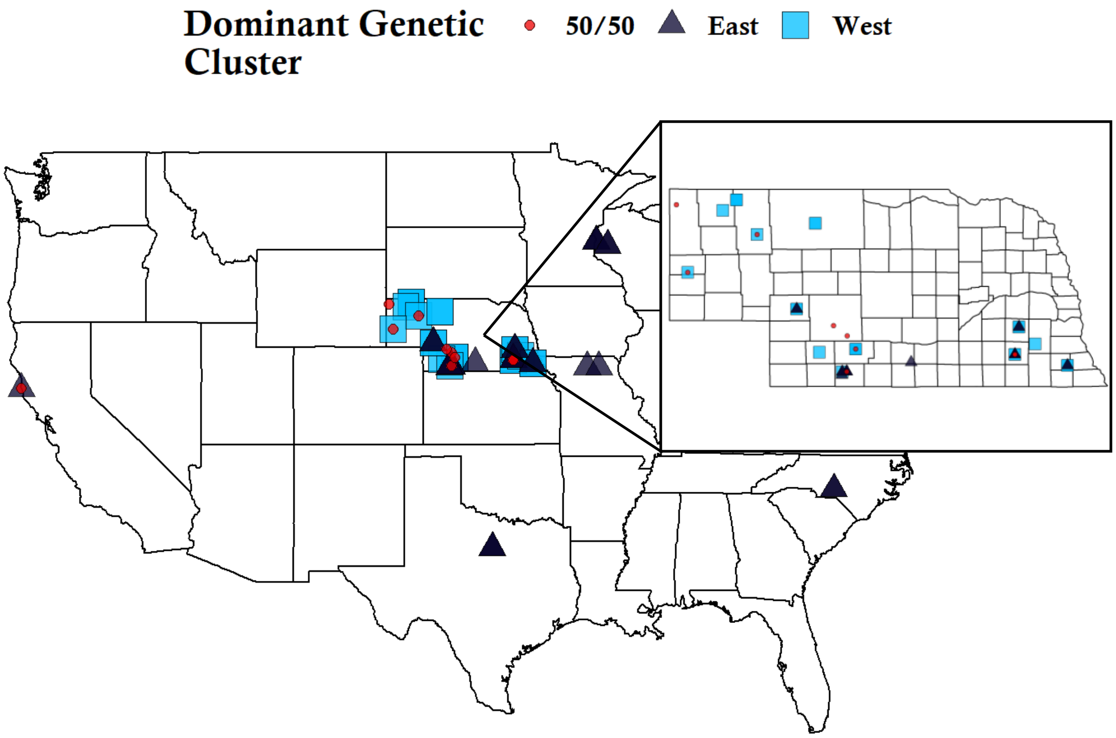


**Fig 1.12 Distribution of East/West Groups**. Map of all individuals with localities marked by whether an individual is made mostly of East or West subspecies. Individuals half East and half West are labeled as red circles in map. Domestics not included.

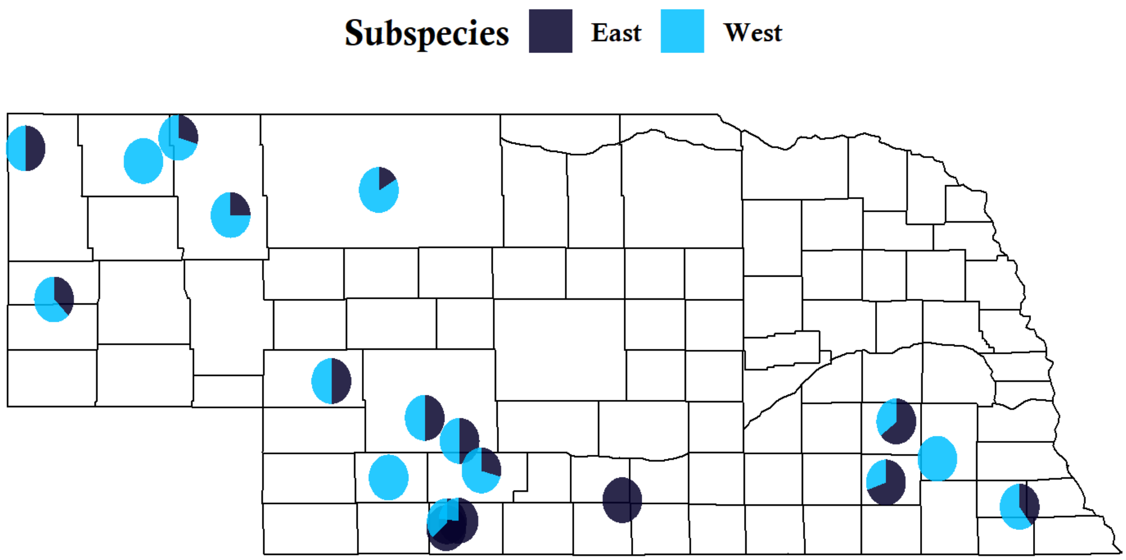


**Fig 1.13 Distribution of East/West Groups in Nebraska**. Map of NE with individuals as pies filled according to total proportion of East or West subspecies diagnostic SNPs within an individual.

## 1.5 DISCUSSION

Overall, my results indicate that there are potentially two genetically and geographically partially distinct groups of wild turkeys instead of the anticipated five. My data indicate that there is a group in the east that consists of the Eastern, Osceola, and Rio Grande subspecies as well as domestics. My data also indicates that there is a distinct group in the west that contains Gould's and Merriam's subspecies. Those turkeys in Nebraska were likely of hybrid ancestry, which corroborate mitogenome results (Vázquez-Miranda et al. in prep.) and limited mtDNA gene results (Speller et al. 2000). My data (Fig 1.4) corroborate previous studies that domestic turkeys could not be distinguished from wild turkeys (Szalanski et al. 2000, Speller et al. 2010), as would be expected from the short amount of time since domestication (Szalanski et al. 2000). My results raise the possibility that the domestic turkeys have an ancestry derived mostly from Eastern, Osceola, and Rio Grande. Speller et al. (2010) suggested two origins of domestic turkeys; in south-west Mexico with *M. g. gallopavo* as the progenitor and the American southwest with Eastern and/or Rio Grande wild turkeys as progenitors. The suggestion of two origins of domestics also corroborates a niche model of wild turkey as the Last Glacial Maximum that shows refugia in the southern and the western US (Fig 1.14) (Vázquez-Miranda et al. in prep.). The discovery of wild turkeys in the tar pits of Rancho La Brea in California (Fragomeni and Prothero 2011) provides support for a possible refugium in the west.

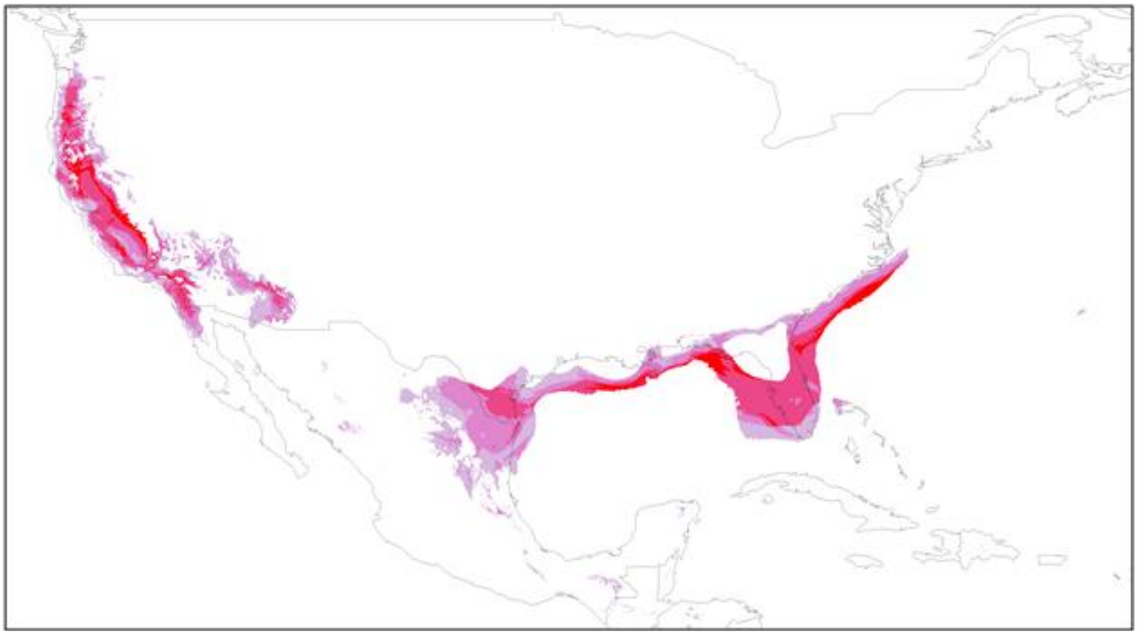

**Fig 1.14 Ecological Niche Model at LGM**. Results of an ecological niche model showing predicted distribution of wild turkey at the Last Glacial Maximum (21,000 years before present), south of the ice sheet and unsuitable habitat. Red shows the areas with highest predicted occurrence. Projected occurrence in areas currently offshore is a result of lowered sea level at the time.

Extensive translocations and release of captive birds could account for the presence of SNPs from all five subspecies in places such as Nebraska, though my results do not align with past studies that found support in nuclear genes for the five subspecies (Mock et al. 2002). A high degree of introgression and all my individuals being hybrids could explain why my STRUCTURE results gave two clusters as the best fit, as subspecies boundaries have been decayed through introgression. Another possibility is that although my SNPs are diagnostic for the 16 specimens chosen to represent each subspecies, they might be shared across other subspecies, which will only be revealed by greater sampling of turkeys across the US. This wider sampling can provide more robust data to better determine if my SNPs are diagnostic. Nonetheless, if the subspecies are genetically distinct to any degree, our molecular data should have recovered more than two distinct groups. Inspection of the 8554 SNPs used to produce the hypothesis of subspecies relationships (Fig. 1.3, 1.15), revealed no synapomorphies for the Eastern and Florida subspecies and subspecies integrity in the tree was a result of an overall average set of relationships. In addition, a phylogenetic tree using all of the sequence (8854 base pairs) that resulted from the amplification of the 11 diagnostic primers, also failed to yield any distinct groups, and therefore could not match subspecies limits (not shown). The presence of Gould and Osceola SNPs in samples from Nebraska and Domestics is unexpected. This could imply that our 11 SNPs are not diagnostic as noted above, or that more SNPs are needed to have the most power for differentiation. Also unexpectedly, the mitogenome and SNP analyses (excluding the full phylogenetic analysis) both suggest that wild turkey in the US can be split into east and west groups. My results have found that in Nebraska all turkeys are hybrids. It also is likely that elsewhere introgression has resulted in erosion of genetic distinction between subspecies. These results are tentative, however, as there are gaps in the data that excluded samples and diagnostic SNPs from being used. Future research should focus on filling these data gaps and obtaining more samples from underrepresented areas.

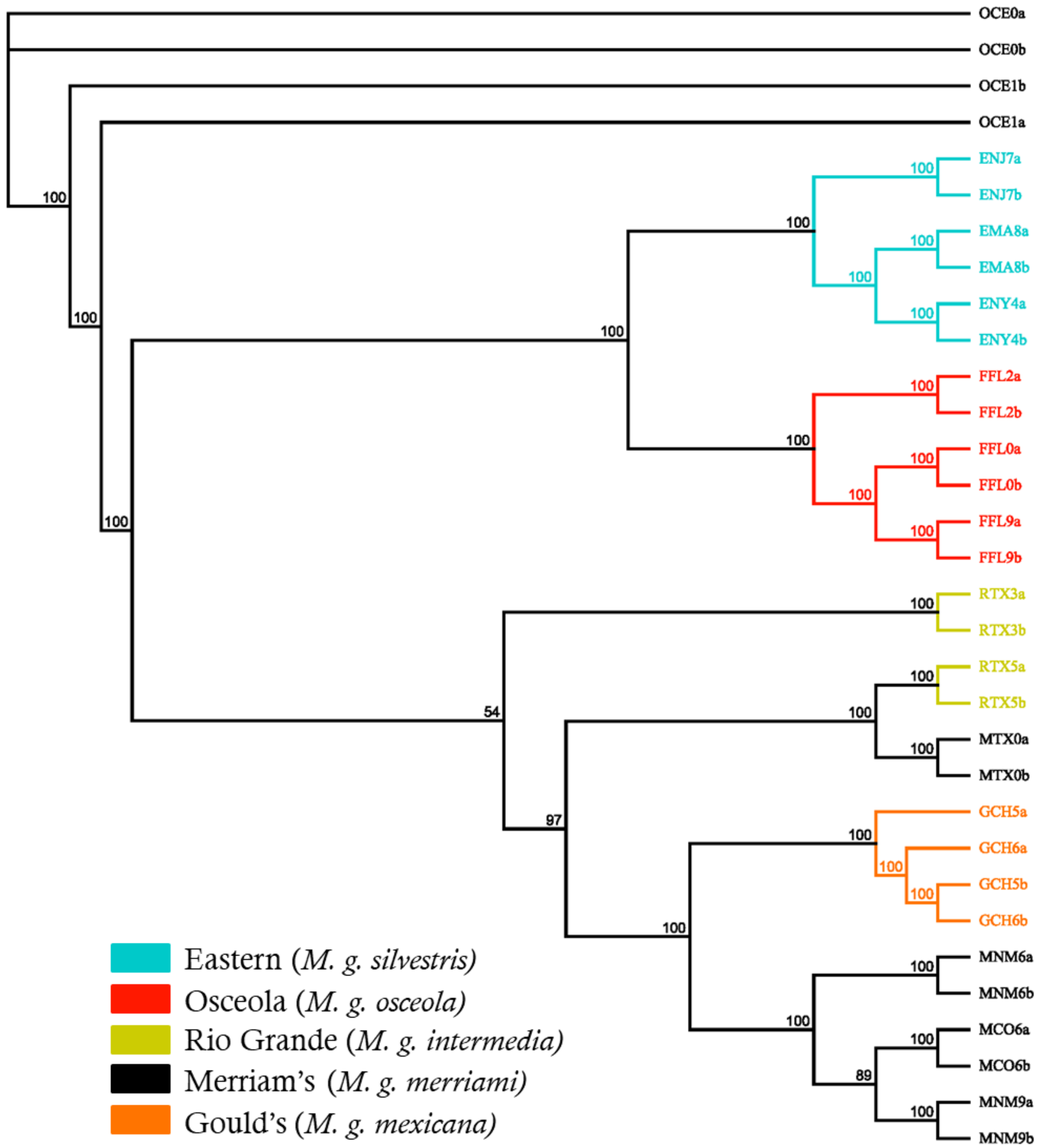


**Fig 1.15 Turkey Whole Genome SNP Phylogeny**. Bayesian phylogeny created from 8554 SNPs from across the turkey genome with phased sequences and oscellated turkey as the outgroup (Vázquez-Miranda et al. in prep.). Created using the Geneious Prime MrBayes plugin with GTR clock model. SNPs were found using a low coverage genome analysis. Each color represents a subspecies and samples are labeled with the first letter as the subspecies designation (O = Oscellated, E = Eastern, G = Gould's, M = Merriam's, and R = Rio Grande) and the next two letters the state abbreviation of a samples locality. Nodes are labeled with posterior probabilities as percentages.

## 1.6 ACKNOWLEDGMENTS

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# CHAPTER 2: TURKEY PLUMAGE COLORATION

## 2.1 ABSTRACT

Correct identification of wildlife species and subspecies by managers is crucial for accomplishing management goals. The wild turkey (*Meleagris gallopavo)* is a useful study system to explore phenotypic and genetic identification of its five morphologically defined subspecies, with whose plumage colorations grade from light to dark. I evaluated whether quantitative coloration data would reveal five geographically distinct spectral ranges that would match the current hypothesized subspecies limits using the putative diagnostic coloration of upper tail covert feather color. I asked hunters to mail 2-3 upper tail covert feathers, which I measured for tip color using a spectrophotometer. I calculated the brightness, chroma, and hue of the spectral data and performed a principal component analysis (PCA) to determine if there were distinct clusters of individuals in the data. The PCA resulted in two distinct reflectance clusters, one from the eastern part of the range and the other from the western portion. Ripley’s K function between the clusters is not significant, which suggests incomplete separation of the two groups. Feather brightness contributes to the separation of the two groups, following a gradient of

higher brightness values in the East and lower brightness values in the West. The PCA analysis does not support the hypothesis of having five distinct spectral ranges that would correspond to traditional subspecies limits and instead suggests two color clusters that roughly follow an East and West delineation. It is possible that today, the transplantations of both wild birds and introduced domestic birds has eroded the color differences that might have once served as diagnostic characteristics of subspecies.

## 2.2 INTRODUCTION

Correct identification of subspecies plays an important role in wildlife management. The accuracy of biodiversity assessments, presence-absence surveys, habitat management plans, population models, and conservation of threatened species can be influenced by incorrect identification of the units of biodiversity, of which subspecies are a part (Zink 2004, Frare et al. 2017). In many instances, genetic techniques can enhance identification of units within species, such as subspecies.

In birds, the wild turkey (*Meleagris gallopavo)* is a useful study system to explore phenotypic and genetic identification of subspecies. Five extant subspecies of wild turkey determined by phenotypes currently range over much of North America: Eastern (*M. g. silvestris*), Osceola (*M. g. osceola*), Rio Grande (*M. g. intermedia*), Merriam's (*M. g. merriami*), and Gould's (*M. g. mexicana*). Although the phenotypes on which subspecies designations have been based have almost certainly been affected by intensive translocations and probably have led to the blending of differences that once were more pronounced. This misidentification can have effects on management decisions, as managers of many states try to cater to hunters that want to harvest certain turkey subspecies.

Upper tail coverts are the main feathers used for turkey subspecies identification. As noted by taxonomists, each subspecies appears to have distinctly colored tips on the upper tail coverts (Aldrich 1967, Hughes and Lee 2015). There is no quantitative evidence, however, that this pattern of variation is currently consistent with historical subspecies boundaries (Appendix A Fig 1.1) I tested whether quantitative analysis of feather coloration matched traditional subspecies limits, and how the pattern of variation compared with that obtained from genetic characters (Chapter 1). That is, I evaluated whether quantitative coloration data would reveal five geographically distinct spectral ranges of upper tail covert feather colors in wild turkeys corresponding to subspecies limits.

The colors of the upper tail coverts are created with melanin pigments that produce darker colors such as black and dark brown. Melanin pigments and iridescence from microstructures are the most familiar colors in turkeys. To produce color, light is absorbed through melanin pigments or reflected from the feather microstructures (Galván, 2011). In addition to colors that humans can see in  the visible light spectrum (~400-700nm), birds are able to see in the ultra-violet (UV) with a total visual range of 300-700nm (Valdez and Benitez-Vieyra, 2016). As humans cannot see in the UV, using instruments that measure UV reflectance is essential for a complete assessment of plumage coloration (Eaton and Lanyon 2003). Therefore, my evaluation of upper tail covert color includes the visual light spectrum including the UV range.

## 2.3 METHODS

### 2.3.1 PLUMAGE SAMPLE AND SPECTRAL DATA COLLECTION

Over the course of the 2018-2019 spring turkey seasons hunters were asked to mail 2-3 upper tail covert feathers. Cooperators were instructed to separate samples by individual, each of which received a unique sample ID. Quantitative color analyses were performed with an Ocean Optics spectrophotometer (USB2000) with a PX-2 light. Two to three randomly chosen samples from each state were chosen to ensure overrepresentation from an area did not bias the results. This resulted in 54 samples from 21 states, with two to three samples per state.

Measurements were taken from two areas of the feather: the matte color tip and the black directly beneath the iridescent band for a control. For each area, a total of five spectral measurements were taken from each feather per sample, resulting in ten to fifteen spectral measurements per individual turkey for both areas (Fig 2.1). Values were recorded and averaged with OOIbase software (830 Douglas Ave., Dunedin, FL, USA 34698). As this device was not well equipped to measure iridescence, I did not measure iridescent patches of feathers.

**Fig 2.1 Feather Measurement Areas.** Example of feather layout for an individual sample, with the feather tip being measured overlaying the black bar of the other feathers. Tip color measured outlined in yellow and black color measured outlined in blue.

Before each measurement was taken, a relative white and dark standard was used to calibrate a 100% reflectance (white) standard and a 0% reflectance (black) standard. The white reference was a Labsphere Diffuse Reflectance Standard, which is a diffused white plastic that is >98% reflective from 250 to 2500 nm and the black standard was a piece of black velvet. At the end of the reflection probe a nonreflective black sleeve was cut in a 45° angle to minimize the specular reflection mismeasurement from white light reaching the sensor (Andersson and Prager 2006). All measurements were recorded in a darkened room to minimize ambient light. Feathers were stacked so that the color band is on the top, with the black bands of the feathers beneath the main feather directly under the color band (Fig 2.1). This layout mimics the natural position of the feathers on a turkey in addition to preventing any spectral contamination from outside of the targeted area. Spectral measurements were first averaged by feather sample and then by individual to get one average measurement for each wavelength per individual (of two to three feathers).

### 2.3.2 SPECTRA STATISTICAL ANALYSIS

Using the R package `pavo` (Maia et al. 2019) in R version 3.6.3 (R Development Core Team, 2020), spectral files were imported into the R workspace for analysis. I calculated the brightness, chroma, and hue of the spectral data, which are parameters that are generally used when quantifying color. Brightness refers to the intensity (i.e. total radiance) of a signal, chroma (i.e. saturation) is the purity of the dominant wavelength, and hue refers to the dominant wavelength of a signal (Fig 2.2). Brightness and chroma are read much like percentages, and they range from 0 – 100. The values for these three parameters were coded and plotted on a map to visualize geographic variation.

After ln-transforming the average spectral measurements, I used the `prcomp` function to perform a principal component analysis (PCA) to determine if there were distinct clusters of individuals in the data. Before performing the analysis I used the `procspec` function from the `pavo` package to normalize the mean values, center the spectra to have a mean reflectance of 0 to remove brightness as a dominant variable, and binned the spectra into 21nm interval bins. Using the `ggbiplot` package (Vu 2011) the first two  principal components were plotted and loading scores on each of the PCs recorded (Hill et al. 2005). To determine if the resulting clusters were significantly random spatially, I used the `dbmss` package (Marcon et al. 2015) to calculate Ripley's K and plot it with global envelopes. Ripley's K is a test of the observed spatial pattern against spatial randomness (CSR).

In addition to analyzing chroma using all wavelengths, I calculated four measures of chroma based on wavelength ranges that describe the predicted spectral sensitivities in domestic turkeys (Hill et al. 2005, Hart et al. 1999). The four chroma measurements are ultra-violet (300-450nm), blue (450-500nm), green (500-550nm), and red (550-700nm). These values were calculated as the proportion of total reflectance occurring between the respective ranges (Hill et al. 2005).

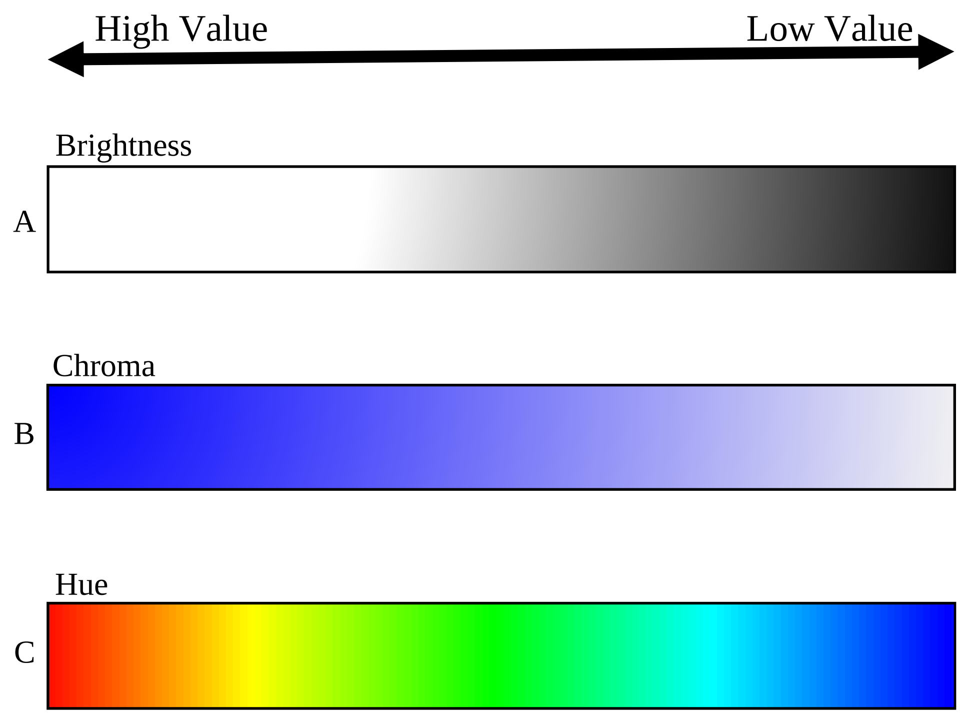


**Fig 2.2 Spectral and color bar representations of brightness, chroma, and hue.** Visual representations of how to discern the 3 most basic colorimetrics of brightness (A), chroma (B), and hue (C). Color bars show only the difference in labeled variable when all others are controlled for. As in, for brightness this shows what the color looks like when chroma and hue are not present etc.

## 2.4 RESULTS

The final data set included much of the historic subspecies' ranges (Fig 2.3). The plot of reflectance values for the 54 individuals showed the highest reflectance value at 35% (Fig 2.4). By plotting my samples on a map colored by the average spectra is in human vision, the higher and lower reflectance values are found over a wide geographic range (Fig 2.5). The exception appears to be in the Northeast US where lower reflectance values dominate.

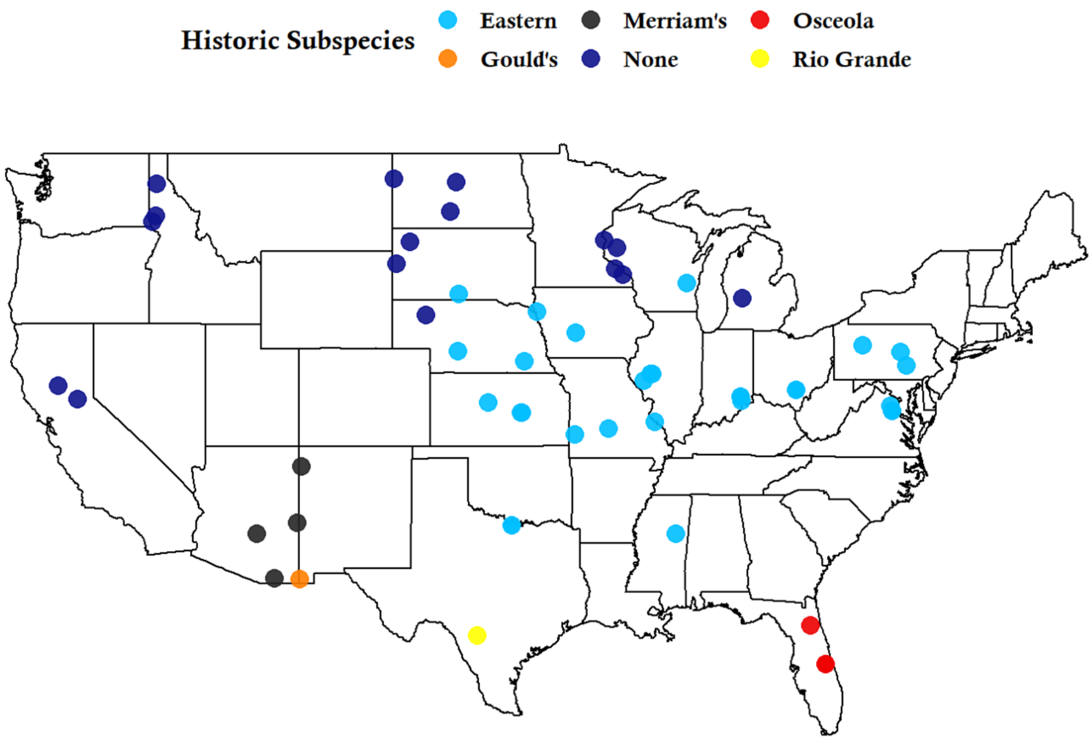


**Fig 2.3 Historic Subspecies Range Sample Representation** Map of where each of the 54 individuals were harvested and sent from. Individuals from California and Idaho represent introduced birds, presumably Rio Grande subspecies. Colored by what the historic subspecies would be for that sample based on Appendix A Fig 1.1.

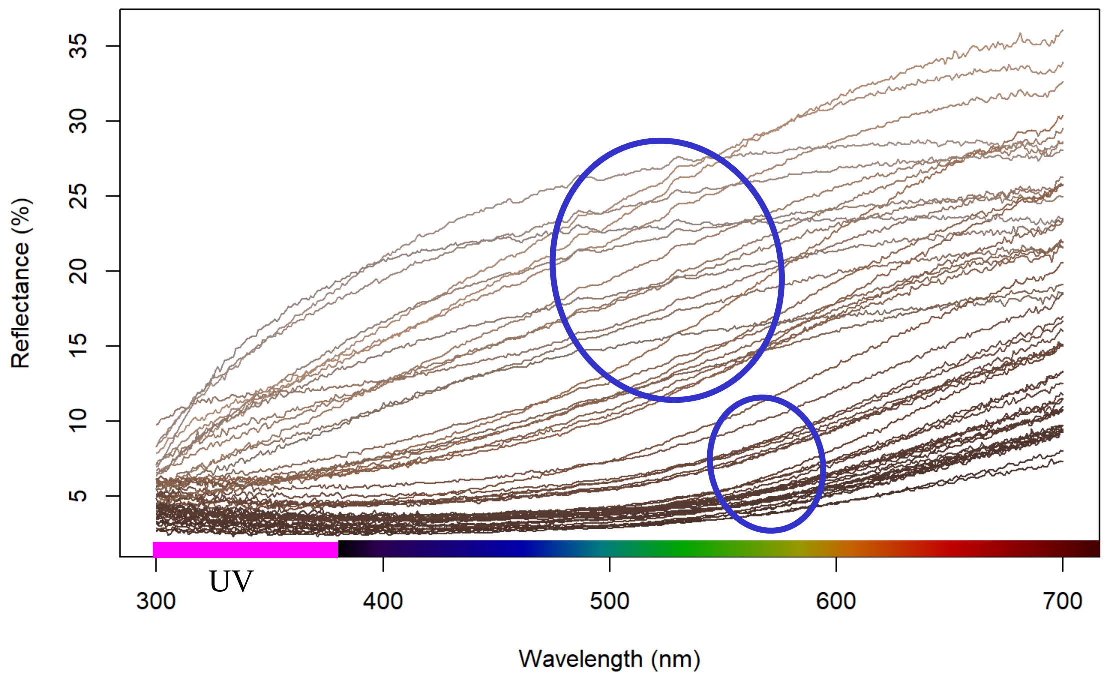


**Fig 2.4 Average spectral reflectance curve for feather tip area.** Contains 54 individuals from 300nm – 700nm, covering the UV and visual light spectrums. Each line represents one individual, and lines are colored by what the color being measured looks like in human vision. Colored bar in bottom right shows the range of the human visual spectrum and UV (purple). Within dotted lines show where the division of two circled distinct groups are visible.

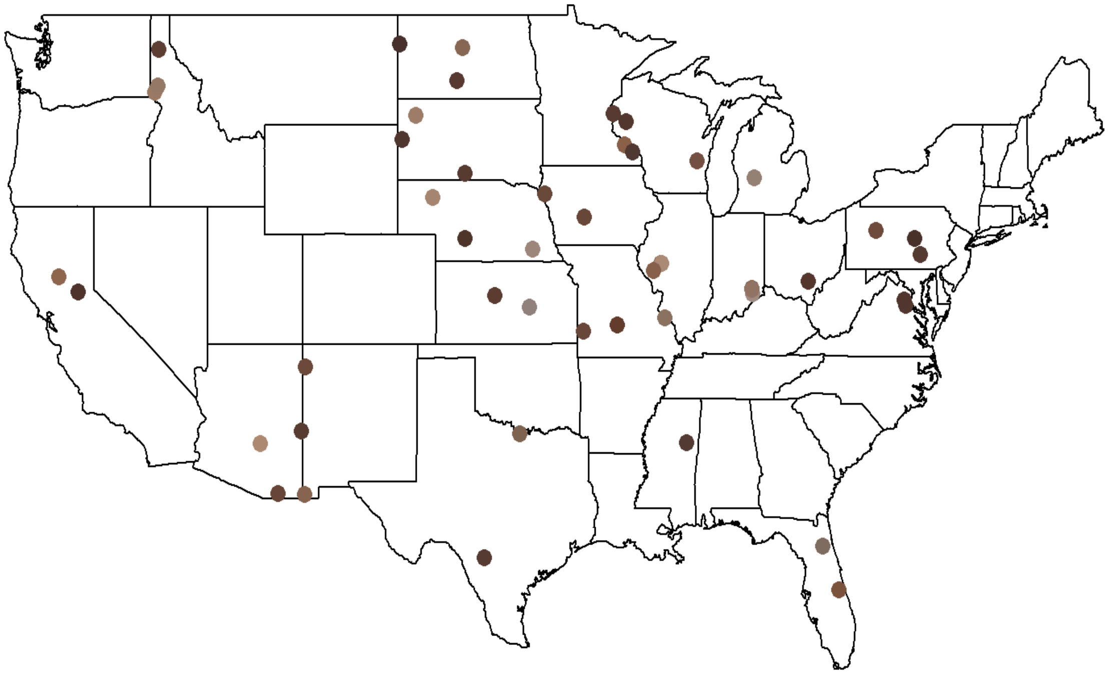

**Fig 2.5 Tip reflectance map.** Locality map of the 54 tip area samples colored by how humans perceive the reflectance value across all wavelengths for all individuals.

The black control results show low average spectral measurements, having a reflectance of less than 10% (Fig 2.6). Most values are between the range of 2-5% reflectance, with a minor number of measurements appearing as outliers most likely due to human error or potential interference from small amounts of iridescence within the black area. Overall, the black area appears to be an acceptable control with its similar reflectance values and geographic heterogeneity.

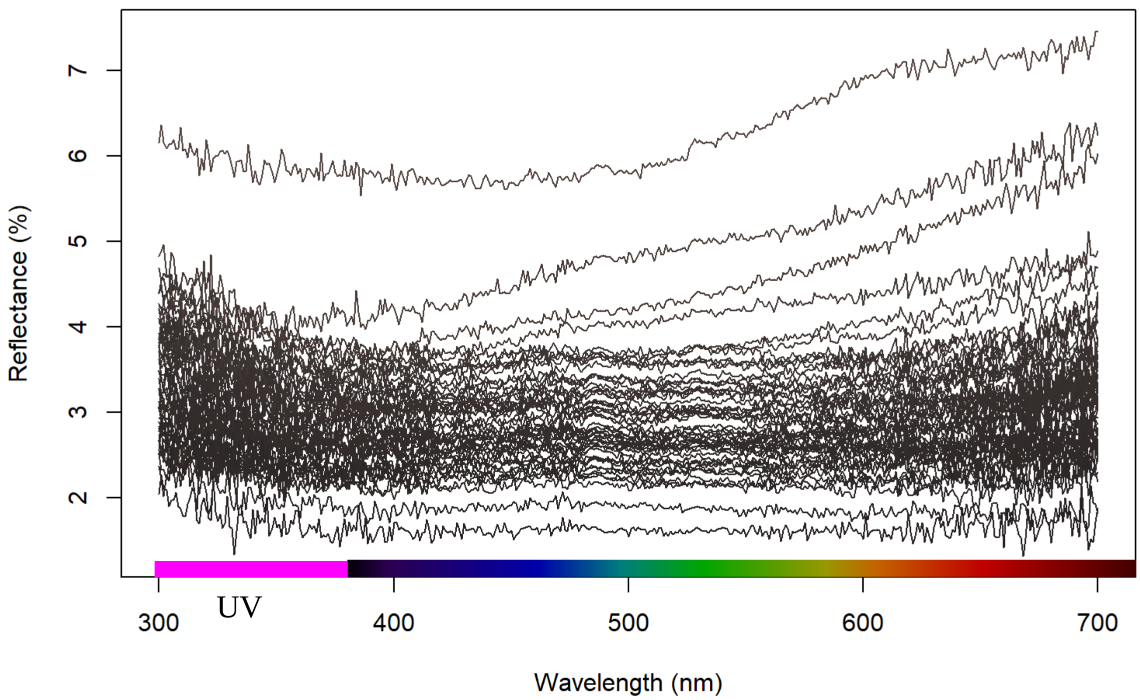


**Fig 2.6 Average spectral reflectance curve for the black feather area.** Contains 54 samples of black area from 300nm – 700nm, covering the UV and visual light spectrums. Each line represents one individual, and lines are colored by what the color being measured looks like in human vision. Colored bar in bottom right shows the range of the human visual spectrum and UV (purple).

### 2.4.1 COLORIMETRICS & PRINCIPAL COMPONENT ANALYSIS

The brightness variable shows a distinction of higher brightness values in the East and lower brightness values in the West (Fig 2.7) and follows the geographic trend from the genetic results in Chapter 1. The highest brightness values were from North Dakota, South Dakota, and Idaho and the lowest values were from Mississippi and Florida. The chroma values appear to closely follow what the human vision colors show us and the hue is consistent in almost all samples at a wavelength of 700 nm (Fig 2.7). The highest chroma value was at 2.1 in Missouri and the lowest value 0.8 in New Mexico

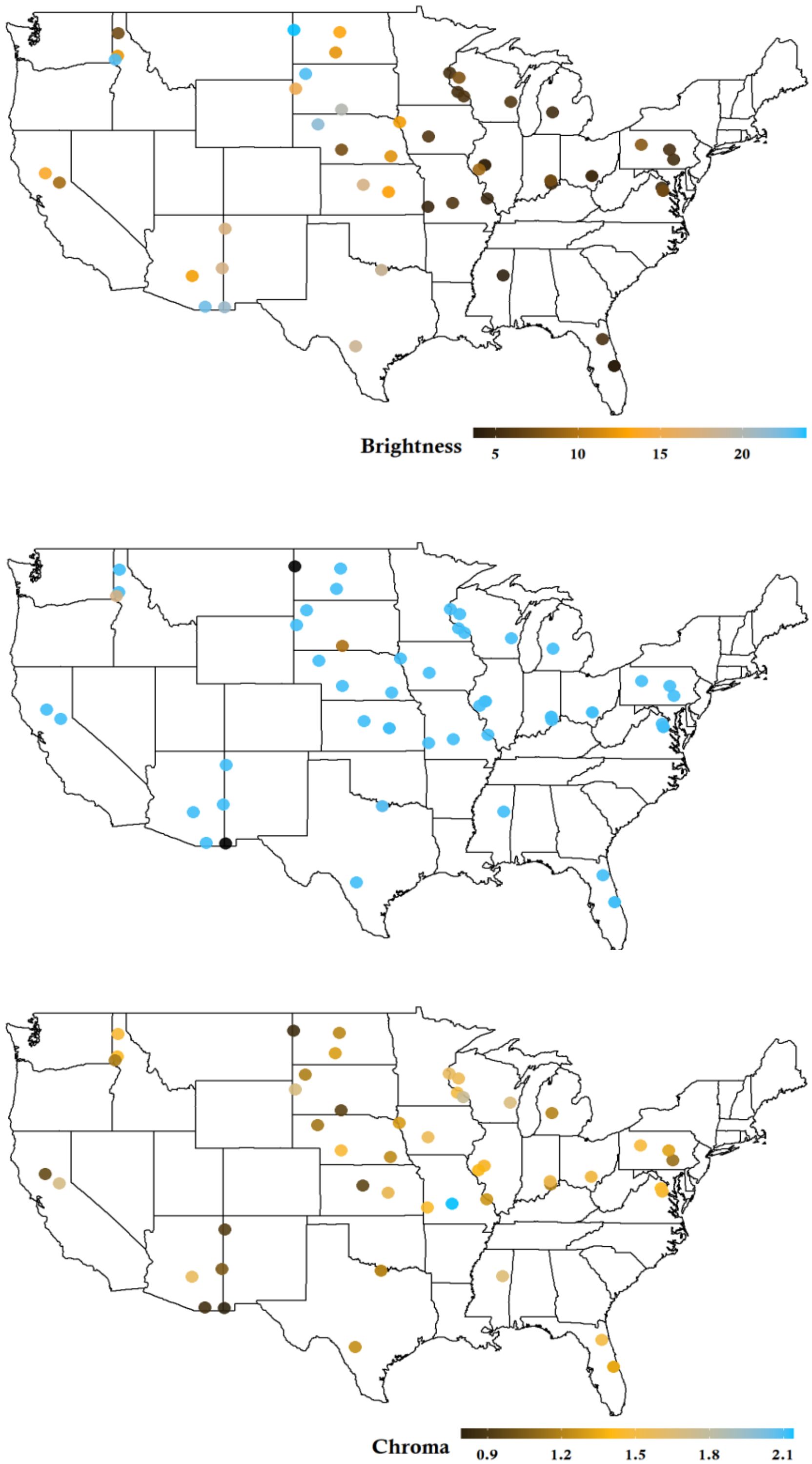


**Fig 2.7 Brightness, saturation, and hue maps for feather tip area.** Locality maps of the 54 feather tip samples showing geographic pattern of brightness (A), saturation (B), and hue (C). The lower these values the darker the point color, the higher these values the lighter the color.

PC1 explained 57.8% of variation in the reflectance spectra of upper tail coverts and PC2 explained 41.2% of the variation (Fig 2.8). For PC1 the wavelengths that influenced where each sample was placed were made up mostly of short and long wavelengths. Wavelengths that were most influential for PC2 were UV and medium wavelengths. The analysis resulted in two distinct reflectance clusters from the data (Fig 2.8), which show one cluster in the East and another in the West (Fig 2.9). From the PCA plot a possible outlier is a sample from Arizona that was assigned to the East cluster in the PCA plot (Fig 2.8). The state of Nebraska predominantly includes the color cluster found in the West (Fig 2.9). By using the K function values plotted against 19 simulations of CSR, I could not reject the null hypothesis of complete spatial randomness of points between the two PCA clusters (2.10) as they overlap within the envelopes.

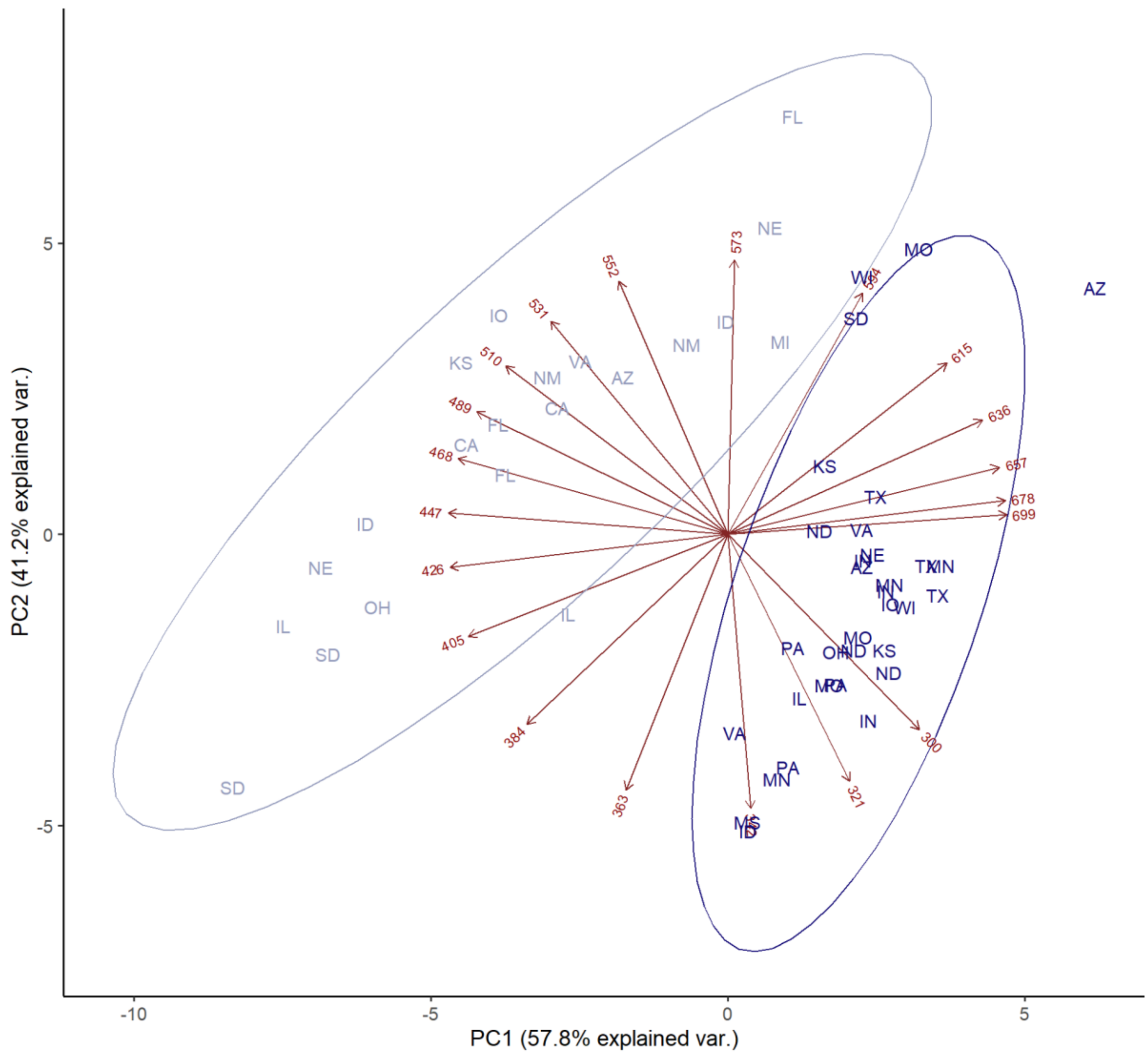


**Fig 2.8 Feather tip PCA plot.** Created from spectral measurements of the 54 individuals with the most supported number of 2 clusters. Individuals are labeled by state they were harvested from.

**Table 2.1 Tip PC Top 10 Loadings.** The wavelengths with the top 10 loading scores for PC1 and PC2, with the wavelength cone type listed

| | **Wavelength** | **Loading Scores** | **Wavelength Type** |
|---|---|---|---|
| PC1 | 447 | -0.2924 | Short |
| | 699 | 0.2918 | Long |
| | 426 | -0.2906 | Short |
| | 678 | 0.2903 | Long |
| | 657 | 0.2839 | Long |
| | 468 | -0.2823 | Short |
| | 405 | -0.2713 | Short |
| | 636 | 0.2666 | Long |
| | 489 | -0.263 | Short |
| | 510 | -0.2323 | Medium |
| PC2 | 573 | 0.0068 | Medium |
| | 342 | 0.0238 | UV |
| | 363 | -0.1067 | UV |
| | 552 | -0.1141 | Medium |
| | 321 | 0.1273 | UV |
| | 594 | 0.1412 | Medium |
| | 531 | -0.1853 | Medium |
| | 300 | 0.2005 | UV |
| | 384 | -0.2102 | Short |
| | 615 | 0.2295 | Long |

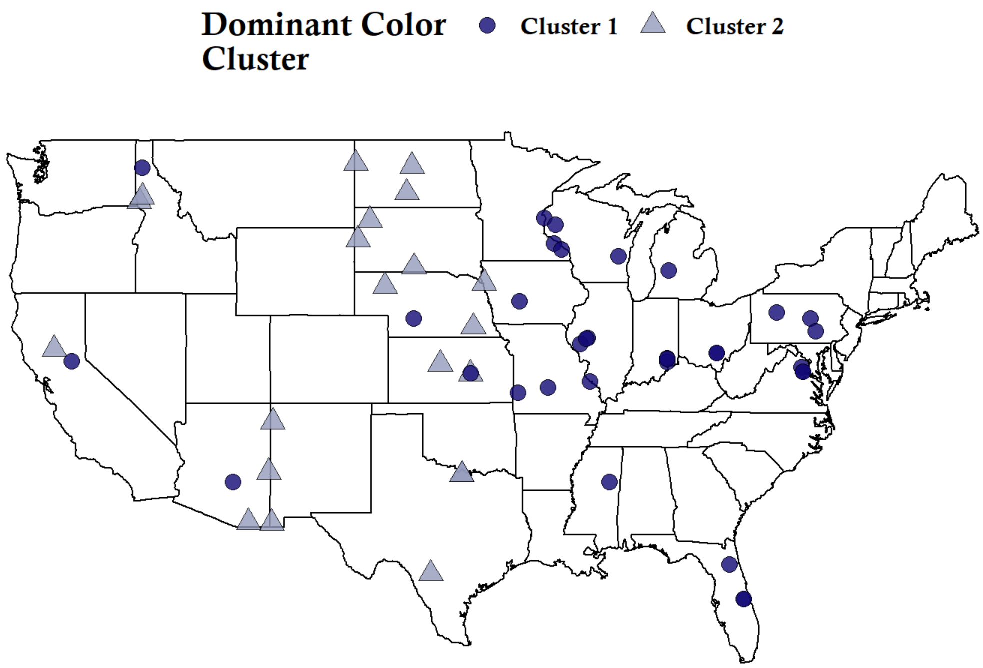


**Fig 2.9 Feather tip PCA Map.** Map of feather samples colored by which PCA cluster the sample was placed in.

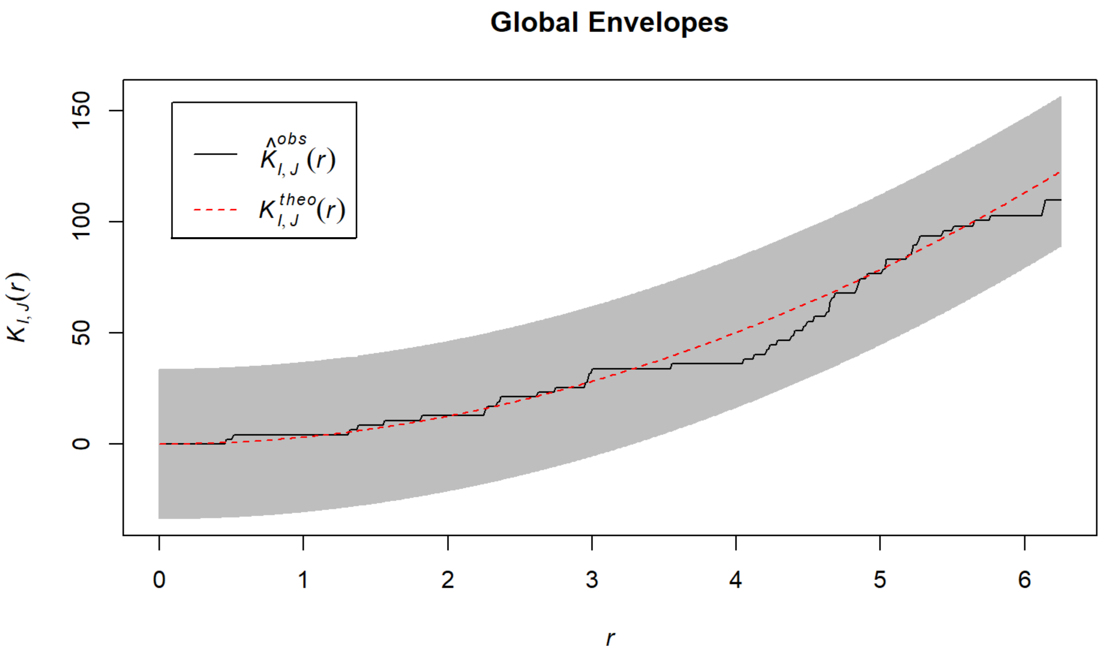


**Fig 2.10 Ripley's K Function Against Complete Spatial Randomness.** Ripley's K function is on the y-axis and distance (r) on the x-axis, the observed K function (black) and the estimated K function at CSR (red). Global envelopes are shaded in

PC1 has a strong significantly positive correlation with brightness (Table 2.1), which may suggest an association with achromatic brightness that consists of black, neutral grays, or white spectra as this is the only variable significant for PC1 (Endler 1990). In comparison PC2 has significant correlations with brightness, saturation, and hue. Contrary to the correlation of PC1 to brightness, PC2 has a moderately negative correlation as well as strong positive correlations with chroma and hue (Table 2.1). The PC plot shows no obvious separation of groups in the UV part of the spectrum.

**Table 2.2 Tip PC Correlations.** Correlations between principal component (PC) scores and colorimetric variables of the 54 feather samples.

| | *Brightness* | *Hue* | *Chroma (All)* | *UV Chroma* | *Blue Chroma* | *Green Chroma* | *Red Chroma* |
|---|---|---|---|---|---|---|---|
| *PC1* | **0.80*** | -0.046 | -0.18 | 0.026 | 0.15 | 0.14 | 0.12 |
| *PC2* | **-0.55*** | **0.66*** | **0.80*** | 0.11 | 0.015 | -0.038 | -0.068 |

*** P < 0.05**

## 2.5 DISCUSSION

The PCA analysis does not support the hypothesis of having five distinct spectral ranges that would correspond to traditional subspecies limits. Instead it suggests two color clusters that roughly follow an East and West delineation, but which are not statistically different. From Ripley's K function it is also not clear if the two clusters are spatially clustered together, as this test merely tells us if we can reject the null of points in each cluster following CSR. According to the loading scores, short and long wavelengths seem to account for most of the variation in the data as these are what the most influential wavelengths from PC1. PC2 has UV and medium wavelengths to account for almost 100% of variation in the data (Table 2.1). Brightness measurements are geographically consistent with my PCA results geographically and Table 2.2 shows that PC1 and PC2 have significant correlations to brightness. This implies that brightness may be a good variable for assigning individuals to the appropriate PCA cluster, especially as human color vision appears to be unreliable. The results also strongly correlate with our SNP results from the previous chapter that suggested two distinct genetic clusters one in the east and one in the west, and the upper tail covert brightness could be an appropriate variable to use to assign genetic clusters as well as color.

Why did the coloration data not corroborate subspecies? The NWTF page (https://www.nwtf.org/hunt/wild-turkey-basics/appearance) shows that the variation in tail bands is more or less continuous, and only by considering the extremes do the subspecies appear distinct. When samples are included from intervening areas, the subspecies characteristics grade into one another, which is typical for many avian subspecies (Zink 2004). It is possible that today, the frequent transplantations of both wild birds and introduced domestic birds has eroded the color differences that might have once served as diagnostic characteristics of subspecies. For example, our samples of wild turkey from California were identified by an experienced hunter as a typical Rio Grande individual. To distinguish subspecies within turkeys could very well require genetic techniques for maximum accuracy, as is the case in many other species. In the Lake Tahoe Basin of the Sierra Nevada in California and Nevada, 4 species of chipmunks whose ranges overlap and are morphologically similar are commonly misidentified, especially between the closer related species. (Frare et al. 2017). In black and white crappie there have been studies showing that phenotypic characteristics are unreliable for distinguishing species and first-generation hybrids (Smith et al. 1995). Even in bacterial species the differentiation of subspecies with phenotypes is difficult where PCR assays have shown that laboratories commonly misidentified them (Hum et al. 1997).

Future research should focus on obtaining a larger sample size per state to provide greater support of our results as well as analyze iridescent plumage. There should also be a focus of analyzing tail fan colors from the visual perspective of turkeys in different age classes, sexes, and molts as well as under various environmental light conditions and ambient light spectrums. DNA extraction from feathers of individuals should also be a priority to allow direct comparison of phenotypes of genotypes of individuals in place of correlative results.

## 2.6 ACKNOWLEDGMENTS

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# CHAPTER 3: EVOLUTION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN MAMMALS

## 3.1 ABSTRACT

Wildlife managers are concerned with transmissible spongiform encephalopathies (TSEs) because they are currently incurable, always fatal, and have the potential to cross species boundaries. Although a wide range of mammals exhibit TSEs, it is currently unclear whether they are evolutionarily clustered or if TSE+ species are randomly distributed phylogenetically. We tested whether mammalian species with TSEs are phylogenetically underdispersed on a phylogenetic tree derived from 102 prion protein gene sequences obtained from the Orthologous Mammalian Markers database. We determined that the PRNP tree was topologically congruent with a species tree for these same 102 taxa constructed from 20 aligned gene sequences, excluding the PRNP sequence. Searches in Google Scholar were done to determine whether a species is known to have expressed a TSE. TSEs were present in a variety of orders excluding Chiroptera, Eulipotyphyla, and Lagomorpha and no marine mammals (Artiodactyla) were recorded to have a TSE. We calculated the phylogenetic signal of binary traits (D-Value) to infer if the phylogenetic distribution of TSEs are conserved or dispersed. The occurrence of TSEs in both trees is non-random (Species tree D-value = 0.291; PRNP tree D-value = 0.273), and appears to have arisen independently in the recent history of different mammalian groups. Our findings suggest that the evolution of TSEs develops in groups of species irrespective of PRNP genotype. The evolution of TSEs merits

continued exploration at a more in-depth phylogenetic level, as well as the search for genetic combinations that might underlie TSE diseases.

## 3.2 INTRODUCTION

Wildlife managers are concerned with transmissible spongiform encephalopathies (TSEs) as they are currently incurable, always fatal, and have the potential to cross species boundaries. Known TSEs include chronic wasting disease (CWD) in cervids, scrapie in sheep, bovine spongiform encephalopathy (BSE, also known as mad cow disease), transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE) and Creutzfeldt-Jakob Disease and Kuru in humans (Collinge and Clarke 2007, Imran and Mahmood 2011, Aguilar-Calvo et al. 2015) . In response to health concerns for livestock and humans, research has focused on learning how species contract TSEs, how they are spread, causes of immunity, and prevention or cures (Osterholm et al. 2019).

Most researchers accept the hypothesis that resistance to TSEs in mammals results from certain genotypes found at the highly conserved prion protein gene (PRNP) (Rongyan et al. 2008) . TSEs are thought to be caused by the misfolding of the host's prion protein (*PrP*) whose primary physiological function is not entirely clear. When correctly folded the prion protein has been theorized to localize at synaptic membranes and be related to normal synaptic functioning, signal transduction, and copper binding (Collinge et al. 1994, Mouillet-Richard 2000, Vassallo and Herms 2003, Roucou and LeBlanc 2005). When misfolded the protein induces other prion proteins to misfold as well, followed by ultimately fatal accumulation in the central nervous system within the host (Rongyan et al. 2008, Harrison et al. 2010, Imran and Mahmood 2011). Misfolded prion proteins can be spontaneously generated (Sigurdson et al. 2009, Osterholm et al. 2019) or introduced to the host by inoculation from the environment or through direct contact with infected individuals (Rongyan et al. 2008, Sigurdson 2008). Differences in mammalian prion proteins might function as species barriers and affect incubation time (Rongyan et al. 2008, Fernández-Borges et al. 2012).

A wide range of mammalian species exhibit TSEs, and it is currently unclear whether they are evolutionarily clustered, or whether TSE+ species are randomly distributed phylogenetically. If a species barrier inhibits horizontal transfer of TSEs, one might predict that related species would exhibit greater susceptibility to TSE expression. The reasoning for this prediction is that phylogenetically more distant relatives would be less similar genetically and, therefore, less susceptible to horizontal (cross-species) transmission.  A phylogenetic test involves constructing a tree from PRNP sequences and testing whether species with TSEs are phylogenetically underdispersed, or clumped within clades (Cavender-Bares et al. 2009). In addition, because the PRNP gene might be under strong selection, it is important to document that the PRNP tree was topologically congruent with one that was not constructed with PRNP data.  If the topology of the two trees differ significantly, it would suggest that selection has constrained the evolution of the PRNP gene.  If the presence of TSEs is phylogenetically clustered, and the two trees are more similar than one would expect by chance, it can be inferred that some lineages are predisposed, perhaps by their genetics, to acquiring this class of diseases.  If TSEs are phylogenetically dispersed, and the two trees are similar, it would suggest that factors other than shared history, such as ecological or behavioral similarities, explain the distribution of TSEs in mammalian taxa. Therefore, we have two objectives: 1) determine if a mammalian species tree and PRNP gene tree have similar topologies and, 2) determine if the presence of TSEs is phylogenetically dispersed in a species tree.

## 3.3 METHODS

We used 102 aligned mammal sequences (Appendix B, Table 3.1) obtained from the Orthologous Mammalian Markers database (OrthoMam) (Ranwez et al. 2007) to construct a phylogenetic hypothesis. In all phylogenic analyses, the platypus (*Ornithorhynchus anatinus*) was used as the outgroup. To determine whether a species is known to have a TSE, searches in Google Scholar were done using the scientific and common name of species combined with “TSE”, “transmissible spongiform encephalopathy”, “prion disease”, “CWD”, “chronic wasting”, “BSE”, “bovine spongiform”, “FSE”, “feline spongiform”, “MSE”, and “mink spongiform”. Specific prion diseases were included in our search to broaden our list of taxa (Appendix B, Table 3.1). Many species appeared to have ambiguous evidence for TSE presence (Appendix B, Table 3.1), and we conservatively scored them as absent. Some species known to express TSEs lacked gene sequences that would have permitted including them in the species tree (e.g., moose (*Alces alces)*, reindeer (*Rangifer tarandus)*, elk (*Cervus canadensis)*, black-tailed deer (*Odocoileus hemonius*)).

In addition to analyzing the aligned sequences available on Orthomam, we computed alternative alignments of the nucleotide data. We aligned sequences three separate ways in the program MEGA X (Kumar et al. 2018): the default MUSCLE settings; the default MUSCLE settings followed with the program Gblocks (Castresana

2000, Talavera and Castresana 2007) under stringent conditions to eliminate poorly aligned positions; and running the available Orthomam alignments only through Gblocks. The different alignment methods produced results equivalent to the aligned Orthomam sequences, which we used in our analysis. In addition, we constructed a phylogenetic tree using sequences of amino acids to determine if particular protein structures were associated with TSE+ species.

Along with species that naturally contract these diseases, our data include species that were shown to express TSEs based on inoculation experiments but at present have no known naturally occurring cases of TSE. To address potential biases in our analyses from the inclusion of these species, we repeated our analyses with only species thought to acquire TSEs naturally. Species which have contracted TSEs from eating BSE infected meat (some felids etc.) were included in our analysis, as we view the consumption of infected tissue a natural pathway of infection susceptibility.

### 3.3.1 PHYLOGENY CONSTRUCTION

To construct a species tree independent of the PRNP gene, we selected 20 aligned gene sequences of coding regions (Appendix B, Table 3.2) for 102 species of mammals spanning 20 orders, 58 families, and 85 genera, and for which evidence of TSE presence/absence was available (Appendix B, Table 3.1). Using the aligned nucleotide coding regions, partitioned (by gene) analyses were run using the Bayesian Evolutionary Analysis by Sampling Trees 2 (BEAST 2) package (Bouckaert et al. 2014). The sequences were analyzed using the best fit model (HKY + G) identified using MEGA X (Kumar et al. 2018). We ran the analyses for 75,000,000 generations while sampling every 5,000 chains under a strict clock model and Yule speciation model. The first 10% of sampled trees were discard as burn-in. Two independent runs were performed with these specifications, and log files were combined in LogCombiner (Drummond and Rambaut 2007) to address low Effective Sample Size (ESS) values of parameters. Resulting trees were re-rooted to the platypus and exported as Nexus and Newick files. The PRNP gene tree was constructed using the same procedures as the species tree, with the best fit model identified as TN93 + G + I. To construct the PRNP gene tree, analyses ran for 10,000,000 generations while sampling every 5,000 chains under a relaxed log normal clock model and Yule model, along with three independent runs that were combined in LogCombiner (Drummond and Rambaut 2007). The phylogenetic analysis of amino acids residues, obtained from Orthomam, followed the same protocol as the two preceding analyses. The amino acid PRNP gene tree had the same specifications as the nucleotide species tree with the best fit model identified as JTT + G and had three independent runs that were combined.

We mapped the presence or absence of TSE on the two trees using stochastic character mapping (Huelsenbeck et al. 2003), which samples character histories based on their posterior probability distribution; we reconstructed the ancestral states using the equal rates model (run in Program R; version 3.5.2, R Development Core Team, 2018). To test if results differed depending on whether species express TSE naturally or only if expression was experimentally induced, we ran two separate analyses. All analyses were done using the R package *phytools* version 0.6-99 (Revell 2012). The function *cophylo* was used to compare the species and gene tree. We also calculated the phylogenetic signal of binary traits (D-value) to infer if the phylogenetic distribution of TSEs are conserved or dispersed (Fritz and Purvis 2010). D-values close to 0 are not randomly distributed and are conserved, whereas values close to 1 are considered randomly distributed on the tree.

## 3.4 RESULTS

### 3.4.1 BASIC GENETIC RESULTS

The number of aligned base pairs ranged from 324 (*Monodelphis domesticus*) to 783 (*Bos taurus*, *Bos mutus*, *Bison bison*), and the total alignment included 861 base pairs, of which 486 were variable. Of the 287 total amino acids (no stop codons were noted), 166 were variable. Nucleotide composition differed little between species with and without TSE (Appendix B, Table 3.3). No amino acid positions consistently separated TSE+ from TSE- species.

### 3.4.2 SPECIES AND PRNP TREES

Most of the internal nodes in the species tree (Fig 3.1) were well supported with posterior probabilities over 0.90, with a few exceptions close to the terminal tips (Fig 3.1). The topology is consistent with current taxonomy, at least to the extent that species from the same orders are supported as clades. In contrast, the PRNP gene tree has relatively few strongly supported nodes (Fig 3.1), although the topology is also consistent with current mammalian ordinal taxonomy. Both trees are topologically congruent, with most of the discrepancies occurring at poorly supported nodes deep in the trees (Fig 3.1). The tree constructed from amino acids (Fig 3.2) is congruent with both the species and PRNP trees.

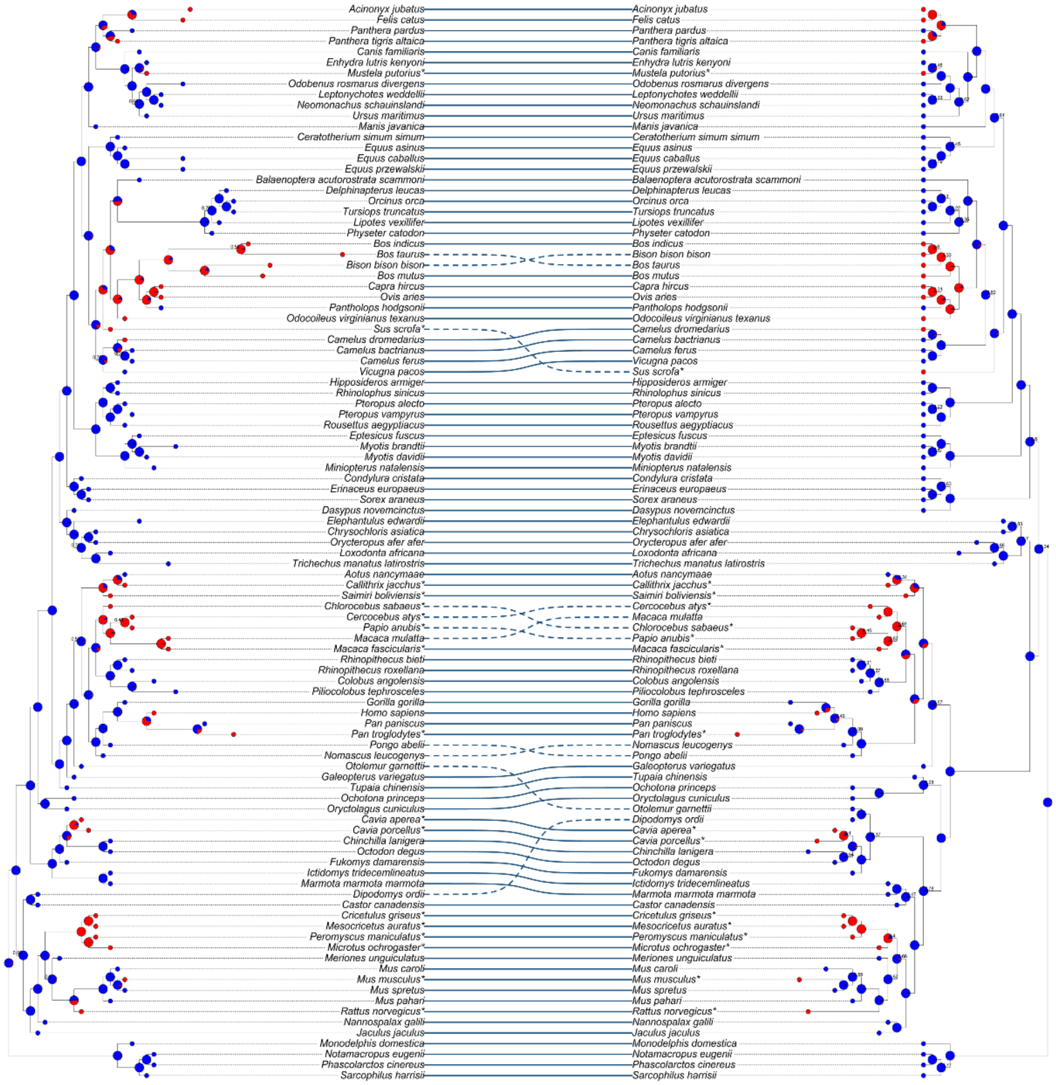

**Fig 3.1. Species Tree (left) and PRNP Gene Tree (right) Comparison using Nucleotides.** Compiled using 20 autosomal genes and rooted with platypus (excluded from figure). Positive TSE presence (red) and absence TSE (blue) shown at tips. Posterior probabilities less than 0.90 and stochastic character mapping probabilities (as pies) displayed at nodes. Congruent tips are connected by solid lines, whereas topological differences between the trees are connected by dashed lines. Species names with asterisks are species which only have records of being TSE+ by inoculation.

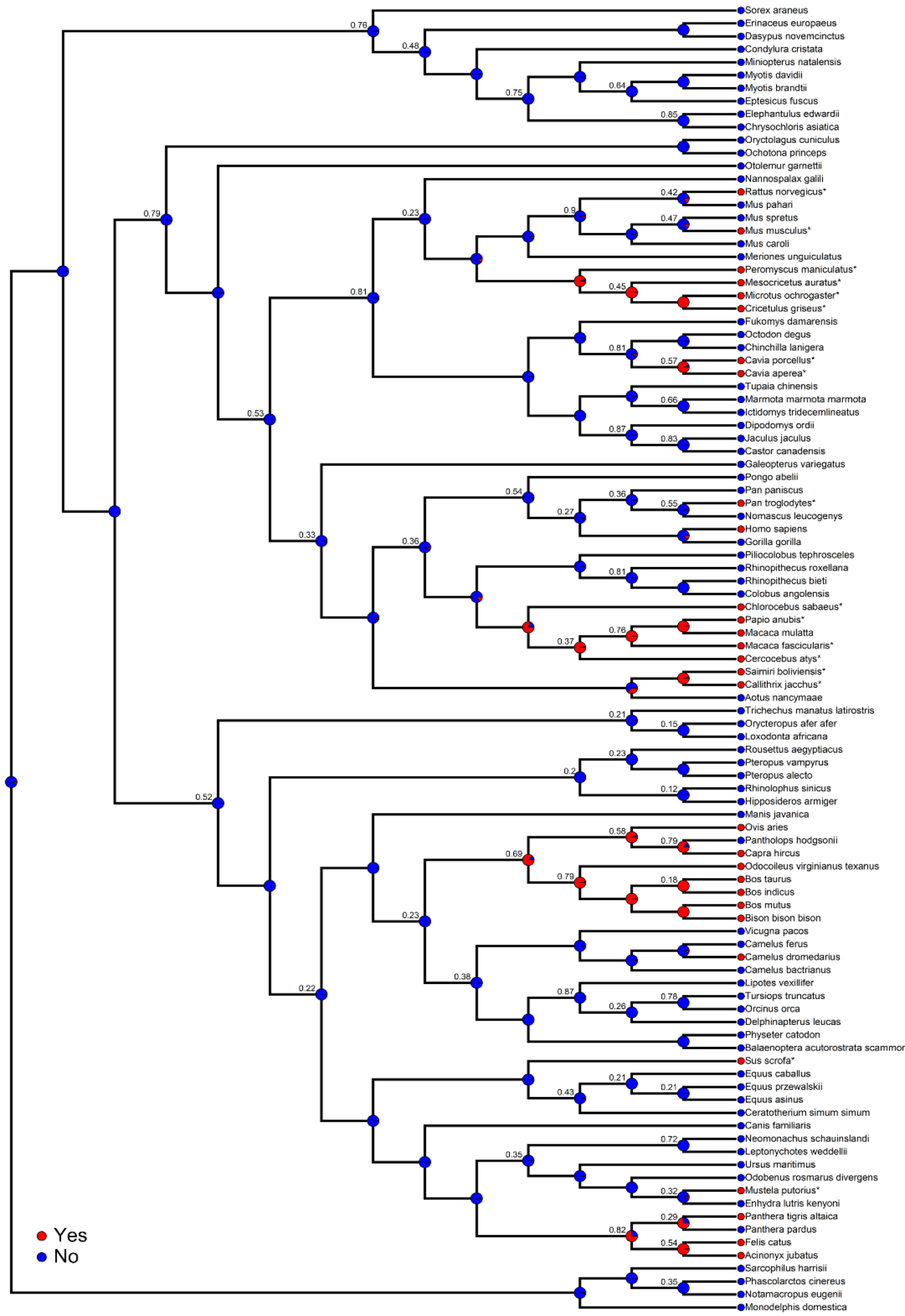


**Fig 3.2. PRNP Gene Tree Created using Amino Acids.** Compiled using the translated PRNP gene and rooted with platypus (excluded from figure). Positive TSE presence (red) and absence TSE (blue) shown at tips. Posterior probabilities less than 0.90 and stochastic character mapping probabilities (as pies) displayed at nodes. Species with only records of being TSE+ by inoculation are denoted by asterisks.

### 3.4.3 RECONSTRUCTION OF TSE EVOLUTION

Because of the congruence of the two trees, we focused on the results from the species tree. TSEs are present in a variety of orders excluding Chiroptera, Eulipotyphyla, and Lagomorpha. No marine mammals (Artiodactyla) have been recorded to have a TSE. According to the ancestral reconstruction, TSEs appear to have arisen relatively recently in TSE+ groups, with the basal condition being absence of TSEs. The reconstruction of TSE evolution is also notable in that there was only one hypothesized transition from TSE presence to absence (Tibetan antelope, *Pantholops hodgsonii*). The presence of TSEs is non-random (D-value = 0.291), suggesting that TSE presence is relatively conserved. Therefore, the distribution of TSEs is not randomly distributed across the phylogeny (Fig. 3.3). The results for the PRNP gene alone were identical to the results inferred from the species tree

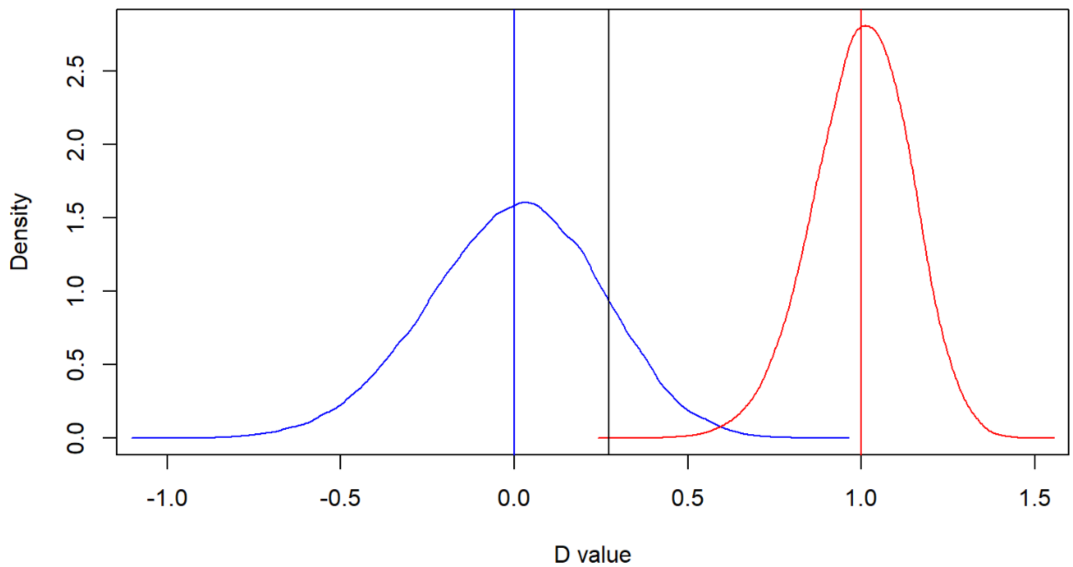


**Fig 3.3. Density plot of scaled observed value of D for the species tree.** The observed value of D for the species tree (D = 0.291) in black compared to simulated values of D = 0 (blue), representing the traits being phylogenetically conserved as expected under a Brownian threshold model (p = 0.115), and D = 1 (red) as the traits being phylogenetically random under a Brownian threshold (p = 0). PRNP tree has similar results (not shown) with observed value of D = 0.273. P = 0.135 for the simulated value of D = 0, and a p = 0 for the simulated value of D = 1.

We removed the 17 species in which TSE presence was experimentally induced (identified with asterisks in Fig 3.1). Even with these removals, our results (Appendix B, Fig 3.4) were identical to those found when they were included in the overall dataset. The species tree D was -0.034, with $p = 0$ for traits being phylogenetically random ($D = 1$) and $p = 0.553$ for traits being phylogenetically conserved ($D = 0$). In the PRNP gene tree the D value was -0.078. $p = 0$ for traits being phylogenetically random ($D = 1$) and $p = 0.594$ for traits being phylogenetically conserved ($D = 0$).

## 3.5 DISCUSSION

Our species tree and the tree inferred solely from the PRNP gene (Fig 3.1) closely match accepted mammalian phylogenetic trees (Murphy et al. 2004, Prasad et al. 2008, Romiguier et al. 2013, Liu et al. 2017, Esselstyn et al. 2017). Therefore, our species tree provides a glimpse into the evolution of TSEs. The occurrence of TSEs is not randomly distributed across the mammal phylogeny. This is true even when species for which TSE presence was only experimentally induced were excluded from the analyses. Therefore, species that are successfully inoculated could be considered when examining the evolution of TSE diseases, as these species possess the physiological capacity to contract TSE diseases. However, many experimental positives involve intracerebral inoculations, which might be too distant from conditions in nature. Furthermore, it is difficult to know from the literature what species were inoculated unsuccessfully and should be scored as resistant.

TSEs appear to have arisen independently and seemingly recently in several major mammalian groups whereas they are absent in others; had information for the 20 genes been available, a group of cervid species (e.g., moose, caribou, elk), all which exhibit TSEs, would have been clustered with *Odocoileus virginianus*. Mawdsley (2020) created a phylogeny that shows that within the family Cervidae the presence of CWD is phylogenetically dispersed, which is consistent with our result at a broader phylogenetic scale.

Another way to examine the evolution of TSE is to determine whether positive or negative species show consistent amino acid substitutions. For example, a particular sequence of amino acids might provide resistance to TSE across many species. We found a lack of amino acid substitutions unique to only TSE+ or TSE- species. However, at the level of individual species, resistance to prion disease is affected by amino acid composition. For example, sheep showing resistance to scrapie have the genotype 136A/154R/171R (Hagenaars et al. 2018). We did not find this genotype in any other mammalian taxa. White-tailed deer exhibiting the 95H or 96S genotypes have a slower progression of CWD than wild type deer (95Q, 96G) (Johnson et al. 2011). It remains to be documented in species that are resistant to prion diseases whether they possess unique amino acid combinations that prevent or delay onset of symptoms.

Given the rarity of known resistant genotypes, it would be surprising to see many instances in which a species evolved resistance. Our ancestral reconstructions included only one instance of a reversal from TSE presence to absence. The nonrandom occurrence of TSEs in some mammalian orders (e.g., rodents, bovids, felines, cervids) suggest that TSEs are a recently evolved class of mammalian disease, which could explain why TSEs are nearly always fatal. Rongyan et al. (2008) suggested that “no dramatic sequence changes have occurred to avoid cross-species TSE infectivity.” Why

TSEs are not more widespread across mammals is unclear at this time. It seems possible that the evolution of TSEs is independent of PRNP genotype.

Given the high fatality rates of TSEs, one might expect strong selection on the PRNP gene. Balancing selection in sheep (Slate 2005) and strong purifying selection for PRNP CDS has been implicated in cattle (Seabury et al. 2004). Humans from Papua New Guinea who survived a kuru epidemic were heterozygous at codon 127 and 129 (Mead et al. 2009). Although prion diseases in humans sometimes emerge after reproduction has occurred, which limits potential for selection, other diseases such as CWD clearly limit reproduction in deer by reducing the reproductive lifespan. In contrast, the congruence between trees reconstructed from nucleotides (Fig 3.1) and amino acid residues (Fig 3.2) suggests that selection has not yet played a major role in the evolution of the PRNP gene. That is, if there were a common PRNP genotype at the amino acid level that conferred resistance to TSEs, those species ought to have been grouped together on the amino acid tree in a way that conflicts with the overall species tree.

Reconstruction of TSE evolution suggests the ancestral state is the absence of TSEs (Fig 3.1), and that certain orders of mammals are apparently at greater risk of developing or contracting these diseases. Alternatively, it is possible that our knowledge of the occurrence of TSEs is incomplete, and one interpretation of our analysis is that all mammalian orders are susceptible, which could be confirmed by more extensive testing. If TSEs are a relatively recent phenomenon in mammals, perhaps enough time has not passed for crossing of species-group barriers. Rongyan et al. (2008) noted that scrapie has been endemic in the United Kingdom for more than 200 years and yet has not crossed the species barrier into humans.

## 3.6 CONCLUSIONS

Understanding how diseases have evolved plays a crucial part in determining which species are currently most at risk. Our findings show that the evolution of TSE+ species is localized, non-random, and develops in groups of species irrespective of a common PRNP genotype. As the PRNP gene has been associated with varying susceptibility to TSE diseases in past studies (Seabury et al. 2004, Sigurdson et al. 2009, Johnson et al. 2011, Acín et al. 2013, Aguilar-Calvo et al. 2014), future studies should focus on other genes. For example, in some cattle breeds and the gayal (*Bos frontalis*), a 23-bp deletion in the PRNP promoter region is associated with susceptibility to bovine spongiform encephalopathy (BSE) (Goldfarb et al. 1991, Memon et al. 2018), although this was not found for white-tailed deer and mule deer (Zink et al. *in review*). Most research into TSEs has involved species such as cows, sheep, deer, rodents, and select primates, which could illuminate how TSEs could cross the species barrier into humans. However, as shown by our list of TSE+ species (Appendix B, Table 3.1) there are many species that are as yet unstudied. Therefore, our list of TSE+ species is likely incomplete due to unavailable information. The evolution of TSEs merits continued exploration at a more in-depth phylogenetic level, as well as the search for genetic combinations that might underlie TSE diseases.

## 3.7 ACKNOWLEDGMENTS

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# APPENDIX A: ADDITIONAL WILD TURKEY GENETIC FIGURES & TABLES

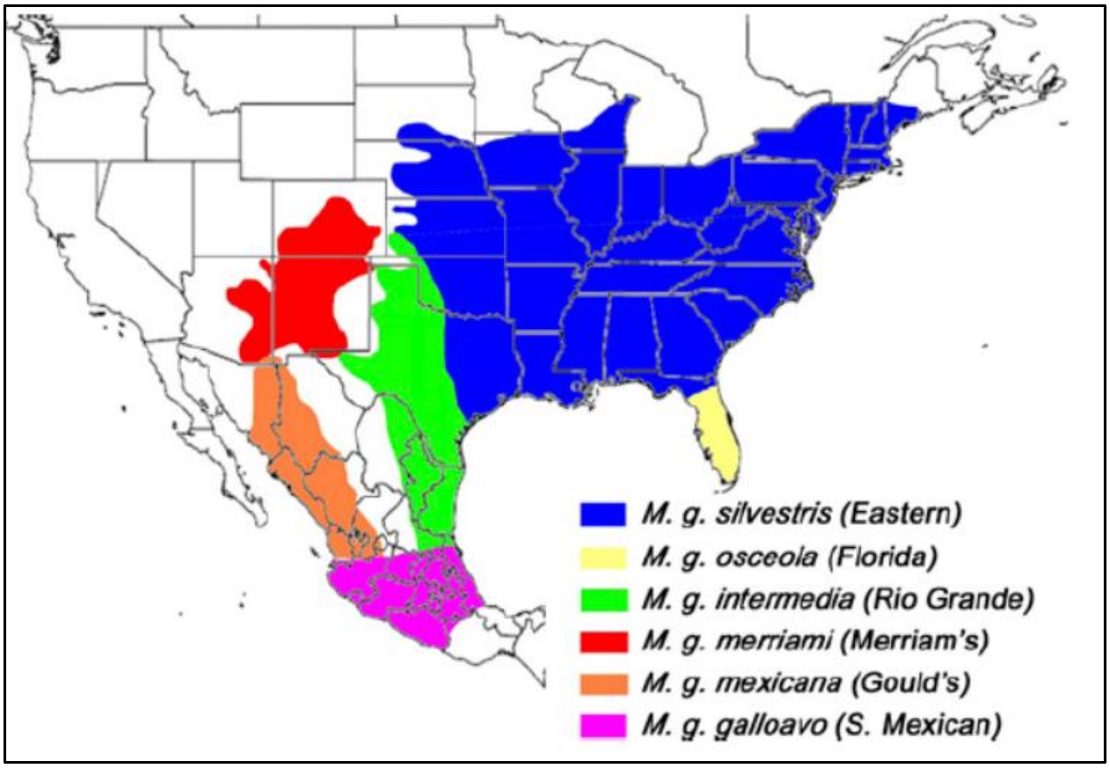


**Fig 1.1 Historic turkey subspecies ranges**. Past wild turkey distribution of 6 wild turkey subspecies before translocations (Schorger 1966). All subspecies are currently extant except M.g.gallopavo.

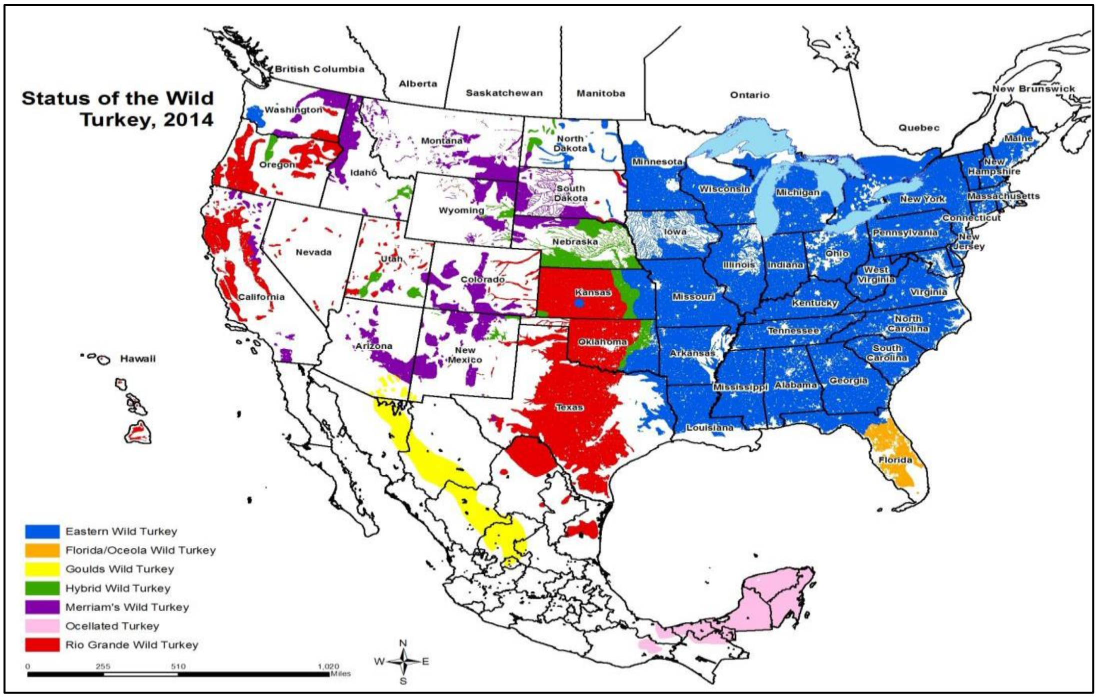


**Fig 1.2 Modern turkey subspecies ranges**. Modern wild turkey distribution in 2014 (from NWTF (2017)). Each color represents a subspecies except the pink, which is the sister species to wild turkey, the ocellated turkey.

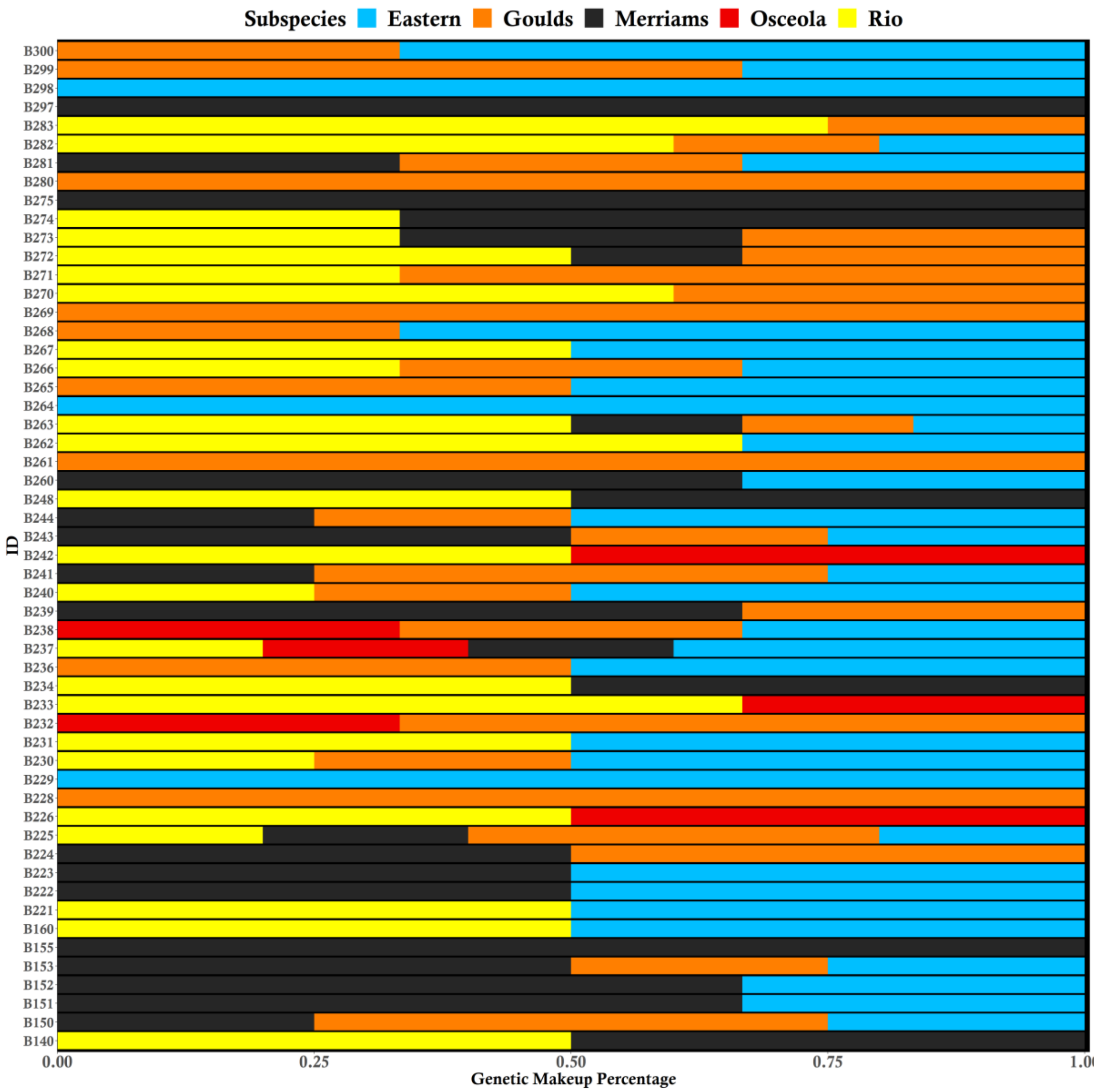


**Fig 1.10 Nebraska SNP Barplot**. Subspecies diagnostic SNP bar plot for 54 samples from NE. Each color represents a subspecies and each bar represents an individual. The length of the color in each bar shows the percentage an individual is made-up of said SNP genetically.

**Table 1.1 Diagnostic SNP Table**. General information on the 11 diagnostic SNPs used for analyses including what chromosome of the turkey genome the SNP can be found, the diagnostic SNP allele, the common SNP allel at the diagnostic position, what position on the reference sequence the diagnostic SNP is located, the immediate alleles surrounding the diagnostic SNP in the reference sequence with the diagnostic SNP highlighted, and the Genbank accession number and range that of the reference sequence used for each SNP.

| *Subspecies* | *Abbreviation* | *Chromosome* | *Diagnostic SNP* | *Common SNP* | *Reference Diagnostic Position* | *Sequence Diagnostic Position* | *Reference Accession* | *Reference Accession Range (bp)* |
|---|---|---|---|---|---|---|---|---|
| *Eastern* | E01 | 17 | T | C | 1095 | TCA**T**CTGC | NC_015027.2 | 3744348-3746537 |
| | E02 | 6 | T | C | 1062 | ACC**C**GCAT | NC_015016.2 | 16907154 - 16909277 |
| *Merriam's* | M01 | 1 | A | G | 999 | GCC**A**GAAT | NC_015011.2 | 43458226 - 43460222 |
| | M04 | Z | G | A | 1435 | ACT**G**GGGT | NC_015041.2 | 53852338 - 53855207 |
| *Rio Grande* | R01 | 4 | T | G | 1481 | CTT**T**GATT | NC_015014.2 | 52859006 - 52861967 |
| | R03 | 15 | T | C | 1560 | GCC**T**TTTC | NC_015025.2 | 15542044 - 15545155 |
| | R04 | 19 | A | G | 922 | GGG**A**GATT | NC_015029.2 | 801137 - 802971 |
| *Osceola* | F02 | 2 | G | A | 1149 | TCT**G**TGTA | NC_015012.2 | 46648002 - 46650299 |
| | F03 | 7 | C | G | 760 | GGT**C**TGGT | NC_015017.2 | 16373463 - 16374982 |
| *Gould's* | G01 | 10 | G | C | 1304 | GGG**G**TCTG | NC_015020.2 | 18656802 - 18659205 |
| | G02 | 11 | G | A | 1058 | GCC**G**GTTTT | NC_015021.2 | 23683607 - 23685720 |

# APPENDIX B: TSE SUPPLEMENTARY MATERIALS

**Table 3.1 List of Species in Data.** List of species with a record of contracting a TSE or not with

| Species | Order | Family | Genus | TSE_present | Induced_TSE_Presence | Reference |
|---|---|---|---|---|---|---|
| Acinonyx_jubatus | Carnivora | Felidae | Acinonyx | Yes | Yes | Sigurdson, C. J., and M. W. Miller (2003). Other animal prion diseases. British Medical Bulletin 66:199–212. |
| Aotus_nancymaae | Primates | Aotidae | Aotus | No | No | |
| Balaenoptera_acutorostrata_scammoni | Artiodactyla | Balaenopteridae | Balaenoptera | No | No | |
| Bison_bison_bison | Artiodactyla | Bovidae | Bison | Yes | Yes | Sigurdson, C. J., and M. W. Miller (2003). Other animal prion diseases. British Medical Bulletin 66:199–212. |
| Bos_indicus | Artiodactyla | Bovidae | Bos | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Bos_mutus | Artiodactyla | Bovidae | Bos | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Bos_taurus | Artiodactyla | Bovidae | Bos | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Callithrix_jacchus | Primates | Callitrichidae | Callithrix | Yes | No | Baker, Ridley, Wells, and Ironside (1998). Prion protein immunohistochemical staining in the brains of monkeys with transmissible spongiform encephalopathy. Neuropathology and Applied Neurobiology 24:476–486. |
| Camelus_bactrianus | Artiodactyla | Camelidae | Camelus | No | No | |
| Camelus_dromedarius | Artiodactyla | Camelidae | Camelus | Yes | Yes | Babelhadj, B., M. A. Di Bari, L. Pirisinu, B. Chiappini, S. B. S. Gaouar, G. Riccardi, S. Marcon, U. Agrimi, R. Nonno, and G. Vaccari (2018). Prion Disease in Dromedary Camels, Algeria. Emerging Infectious Diseases 24:1029–1036. |
| Camelus_ferus | Artiodactyla | Camelidae | Camelus | No | No | |
| Canis_familiaris | Carnivora | Canidae | Canis | No | No | |
| Capra_hircus | Artiodactyla | Bovidae | Capra | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Castor_canadensis | Rodentia | Castoridae | Castor | No | No | |
| Cavia_aperea | Rodentia | Caviidae | Cavia | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Cavia_porcellus | Rodentia | Caviidae | Cavia | Yes | No | Watts, J. C., K. Giles, D. J. Saltzberg, B. N. Dugger, S. Patel, A. Oehler, S. Bhardwaj, A. Sali, and S. B. Prusiner (2016). Guinea Pig Prion Protein Supports Rapid Propagation of Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease Prions. Journal of Virology 90:9558–9569. |
| Ceratotherium_simum _simum | Perissodactyla | Rhinocerotidae | Ceratotherium | No | No | |
| Cercocebus_atys | Primates | Cercopithecidae | Cercocebus | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Chinchilla_lanigera | Rodentia | Chinchillidae | Chinchilla | No | No | |
| Chlorocebus_sabaeus | Primates | Cercopithecidae | Chlorocebus | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Chrysochloris_asiatica | Afrosoricida | Chrysochloridae | Chrysochloris | No | No | |
| Colobus_angolensis | Primates | Cercopithecidae | Colobus | No | No | |
| Condylura_cristata | Eulipotyphla | Talpidae | Condylura | No | No | |
| Cricetulus_griseus | Rodentia | Cricetidae | Cricetulus | Yes | No | Kimberlin R. H., Cole S. & Walker C. A. (1986) Neuropathology and Applied Neurobiology 12, 197–206 |
| Dasypus_novemcinctus | Cingulata | Dasypodidae | Dasypus | No | No | |
| Delphinapterus_leucas | Artiodactyla | Monodontidae | Delphinapterus | No | No | |
| Dipodomys_ordii | Rodentia | Heteromyidae | Dipodomys | No | No | |
| Elephantulus_edwardii | Macroscelidea | Macroscelididae | Elephantulus | No | No | |
| Enhydra_lutris_kenyoni | Carnivora | Mustelidae | Enhydra | No | No | |
| Eptesicus_fuscus | Chiroptera | Vespertilionidae | Eptesicus | No | No | |
| Equus_asinus | Perissodactyla | Equidae | Equus | No | No | |
| Equus_caballus | Perissodactyla | Equidae | Equus | No | No | |
| Equus_przewalskii | Perissodactyla | Equidae | Equus | No | No | |
| Erinaceus_europaeus | Eulipotyphla | Erinaceidae | Erinaceus | No | No | |
| Felis_catus | Carnivora | Felidae | Felis | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Fukomys_damarensis | Rodentia | Bathyergidae | Fukomys | No | No | |
| Galeopterus_variegatus | Dermoptera | Cynocephalidae | Galeopterus | No | No | |
| Gorilla_gorilla | Primates | Hominidae | Gorilla | No | No | |
| Hipposideros_armiger | Chiroptera | Hipposideridae | Hipposideros | No | No | |
| Homo_sapiens | Primates | Hominidae | Homo | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Ictidomys_tridecemlineatus | Rodentia | Sciuridae | Ictidomys | No | No | |
| Jaculus_jaculus | Rodentia | Dipodidae | Jaculus | No | No | |
| Leptonychotes_weddellii | Carnivora | Phocidae | Leptonychotes | No | No | |
| Lipotes_vexillifer | Artiodactyla | Lipotidae | Lipotes | No | No | |
| Loxodonta_africana | Proboscidea | Elephantidae | Loxodonta | No | No | |
| Macaca_fascicularis | Primates | Cercopithecidae | Macaca | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Macaca_mulatta | Primates | Cercopithecidae | Macaca | Yes | Yes | Sigurdson, C. J., and M. W. Miller (2003). Other animal prion diseases. British Medical Bulletin 66:199–212. |
| Manis_javanica | Pholidota | Manidae | Manis | No | No | |
| Marmota_marmota _marmota | Rodentia | Sciuridae | Marmota | No | No | |
| Meriones_unguiculatus | Rodentia | Muridae | Meriones | No | No | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mesocricetus_auratus | Rodentia | Cricetidae | Mesocricetus | Yes | No | Marsh, R. F., D. Burger, R. Eckroade, G. M. Zu, and R. P. Hanson (1969). A Preliminary Report on the Experimental Host Range of the Transmissible Mink Encephalopathy Agent. Journal of Infectious Diseases 120:713–719. |
| Microtus_ochrogaster | Rodentia | Cricetidae | Microtus | Yes | No | Kurt, T. D., D. M. Seelig, J. R. Schneider, C. J. Johnson, G. C. Telling, D. M. Heisey, and E. A. Hoover (2011). Alteration of the Chronic Wasting Disease Species Barrier by In Vitro Prion Amplification. Journal of Virology 85:8528–8537. |
| Miniopterus_natalensis | Chiroptera | Miniopteridae | Miniopterus | No | No | |
| Monodelphis_domestica | Didelphimorphia | Didelphidae | Monodelphis | No | No | |
| Mus_caroli | Rodentia | Muridae | Mus | No | No | |
| Mus_musculus | Rodentia | Muridae | Mus | Yes | No | Barlow, R. M. (1972). Transmissible mink encephalopathy: pathogenesis and nature of the aetiological agent. Journal of Clinical Pathology 25:102–109. |
| Mus_pahari | Rodentia | Muridae | Mus | No | No | |
| Mus_spretus | Rodentia | Muridae | Mus | No | No | |
| Mustela_putorius | Carnivora | Mustelidae | Mustela | Yes | No | Bartz, J. C., R. F. Marsh, D. I. McKenzie, and J. M. Aiken (1998). The Host Range of Chronic Wasting Disease Is Altered on Passage in Ferrets. Virology 251:297–301. |
| Myotis_brandtii | Chiroptera | Vespertilionidae | Myotis | No | No | |
| Myotis_davidii | Chiroptera | Vespertilionidae | Myotis | No | No | |
| Nannospalax_galili | Rodentia | Spalacidae | Nannospalax | No | No | |
| Neomonachus _schauinslandi | Carnivora | Phocidae | Neomonachus | No | No | |
| Nomascus_leucogenys | Primates | Hylobatidae | Nomascus | No | No | |
| Notamacropus_eugenii | Diprotodontia | Macropodidae | Notamacropus | No | No | |
| Ochotona_princeps | Lagomorpha | Ochotonidae | Ochotona | No | No | |
| Octodon_degus | Rodentia | Octodontidae | Octodon | No | No | |
| Odobenus_rosmarus _divergens | Carnivora | Odobenidae | Odobenus | No | No | |
| Odocoileus_virginianus _texanus | Artiodactyla | Cervidae | Odocoileus | Yes | Yes | Sigurdson, C. J. (2008). A prion disease of cervids: Chronic wasting disease. Veterinary Research 39:41. |
| Orcinus_orca | Cetacea | Delphinidae | Orcinus | No | No | |
| Ornithorhynchus_anatinus | Monotremata | Ornithorhynchidae | Ornithorhynchus | No | No | |
| Orycteropus_afer_afer | Tubulidentata | Orycteropodidae | Orycteropus | No | No | |
| Oryctolagus_cuniculus | Lagomorpha | Leporidae | Oryctolagus | No | No | |
| Otolemur_garnettii | Primates | Galagidae | Otolemur | No | No | |
| Ovis_aries | Artiodactyla | Bovidae | Ovis | Yes | Yes | Imran, M., and S. Mahmood (2011). An overview of animal prion diseases. Virology Journal 8. |
| Pan_paniscus | Primates | Hominidae | Pan | No | No | |
| Pan_troglodytes | Primates | Hominidae | Pan | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Panthera_pardus | Carnivora | Felidae | Panthera | No | No | |
| Panthera_tigris_altaica | Carnivora | Felidae | Panthera | Yes | Yes | Sigurdson, C. J., and M. W. Miller (2003). Other animal prion diseases. British Medical Bulletin 66:199–212. |
| Pantholops_hodgsonii | Artiodactyla | Bovidae | Pantholops | No | No | |
| Papio_anubis | Primates | Cercopithecidae | Papio | Yes | No | Masters, C. L., M. P. Alpers, D. C. Gajdusek, C. J. Gibbs jr., and B. A. Kakulas (1976). Experimental Kuru in the Gibbon and Sooty Mangabey and Creutzfeldt-Jakob Disease in the Pigtailed Macaque. Journal of Medical Primatology 5:205–209. |
| Peromyscus_maniculatus | Rodentia | Cricetidae | Peromyscus | Yes | No | Kurt, T. D., D. M. Seelig, J. R. Schneider, C. J. Johnson, G. C. Telling, D. M. Heisey, and E. A. Hoover (2011). Alteration of the Chronic Wasting Disease Species Barrier by In Vitro Prion Amplification. Journal of Virology 85:8528–8537. |
| Phascolarctos_cinereus | Diprotodontia | Phascolarctidae | Phascolarctos | No | No | |
| Physeter_catodon | Artiodactyla | Physeteridae | Physeter | No | No | |
| Piliocolobus_tephrosceles | Primates | Cercopithecidae | Piliocolobus | No | No | |
| Pongo_abelii | Primates | Hominidae | Pongo | No | No | |
| Pteropus_alecto | Chiroptera | Pteropodidae | Pteropus | No | No | |
| Pteropus_vampyrus | Chiroptera | Pteropodidae | Pteropus | No | No | |
| Rattus_norvegicus | Rodentia | Muridae | Rattus | Yes | No | Zlotnik, I., and Rennie, J. C. (1965). Experimental transmission of mouse passaged scrapie to goats, sheep, rats and hamsters. J. conap. Path., 75, 147-157. |
| Rhinolophus_sinicus | Chiroptera | Rhinolophidae | Rhinolophus | No | No | |
| Rhinopithecus_bieti | Primates | Cercopithecidae | Rhinopithecus | No | No | |
| Rhinopithecus_roxellana | Primates | Cercopithecidae | Rhinopithecus | No | No | |
| Rousettus_aegyptiacus | Chiroptera | Pteropodidae | Rousettus | No | No | |
| Saimiri_boliviensis | Primates | Cebidae | Saimiri | Yes | No | ECKROADE, R. J., ZU RHEIN, G. M., MARSH, R. F. & HANSON, R. P. (1970). Transmissible mink encephalopathy: experimental transmission to the squirrel monkey. Science 169, 1088-1090. |
| Sarcophilus_harrisii | Dasyuromorphia | Dasyuridae | Sarcophilus | No | No | |
| Sorex_araneus | Eulipotyphla | Soricidae | Sorex | No | No | |
| Sus_scrofa | Artiodactyla | Suidae | Sus | Yes | No | Wells, G. A. H. (2003). Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs. Journal of General Virology 84:1021–1031. |
| Trichechus_manatus _latirostris | Sirenia | Trichechidae | Trichechus | No | No | |
| Tupaia_chinensis | Scandentia | Tupaiidae | Tupaia | No | No | |
| Tursiops_truncatus | Cetacea | Delphinidae | Tursiops | No | No | |
| Ursus_maritimus | Carnivora | Ursidae | Ursus | No | No | |
| Vicugna_pacos | Artiodactyla | Camelidae | Vicugna | No | No | |

**Table 3.2 Genes used to Construct Phylogenies** List of genes used for BEAST analysis, all gene sequences obtained from Orthomam.

| *Gene* | *Full Name* | *Sites* | *Gene Function* |
|---|---|---|---|
| *AGPS* | Alkylglycerone phosphate synthase | 1983 | protein binding, transferase activity |
| *ATXN2* | ataxin 2 | 3216 | Protein coding |
| *C9orf72* | C9orf72-SMCR8 complex subunit | 1443 | Protein coding |
| *DENND6A* | DENN domain containing 6A | 1827 | Protein coding |
| *EPC2* | enhancer of polycomb homolog 2 | 2427 | Protein coding |
| *FBXO47* | F-box protein 47 | 1365 | Protein coding |
| *FOXJ3* | forkhead box J3 | 1881 | Protein coding |
| *LRRC40* | leucine rich repeat containing 40 | 1812 | Protein coding |
| *MBIP* | MAP3K12 binding inhibitory protein 1 | 1032 | Protein coding |
| *ORC4* | origin recognition complex subunit 4 | 1308 | Protein coding |
| *PARPBP* | PARP1 binding protein | 1740 | Protein coding |
| *PTBP2* | polypyrimidine tract binding protein 2 | 1596 | Protein coding |
| *RB1* | RB transcriptional corepressor 1 | 2793 | Protein coding |
| *SLC39A6* | solute carrier family 39 member 6 | 2343 | Protein coding |
| *STXBP3* | syntaxin binding protein 3 | 1788 | Protein coding |
| *SUCO* | SUN domain containing ossification factor | 3669 | Protein coding |
| *VPS54* | VPS54 subunit of GARP complex | 2934 | Protein coding |
| *YIPF4* | Yip1 domain family member 4 | 738 | Protein coding |
| *ZC3H6* | zinc finger CCCH-type containing 6 | 3573 | Protein coding |
| *ZCCHC11* | terminal uridylyl transferase 4 | 4998 | Protein coding |

**Table 3.3 PRNP Nucleotide Composition** Nucleotide composition for the PRNP gene averaged by TSE+ and TSE- species

| *Nucleotide frequencies per codon position* | *Average for TSE+ species* | *Average for TSE- species* |
|---|---|---|
| *T-1* | 14.6983878 | 14.646186 |
| *C-1* | 24.3504366 | 23.9659998 |
| *A-1* | 27.2304251 | 27.4319861 |
| *G-1* | 33.7207505 | 33.9558282 |
| *T-2* | 21.8791067 | 21.7368085 |
| *C-2* | 17.7835228 | 17.7451334 |
| *A-2* | 30.5391589 | 30.8195962 |
| *G-2* | 29.7982116 | 29.6984619 |
| *T-3* | 19.74754 | 17.6817235 |
| *C-3* | 38.1539884 | 40.7078425 |
| *A-3* | 12.5026766 | 11.1711142 |
| *G-3* | 29.5957951 | 30.4393198 |

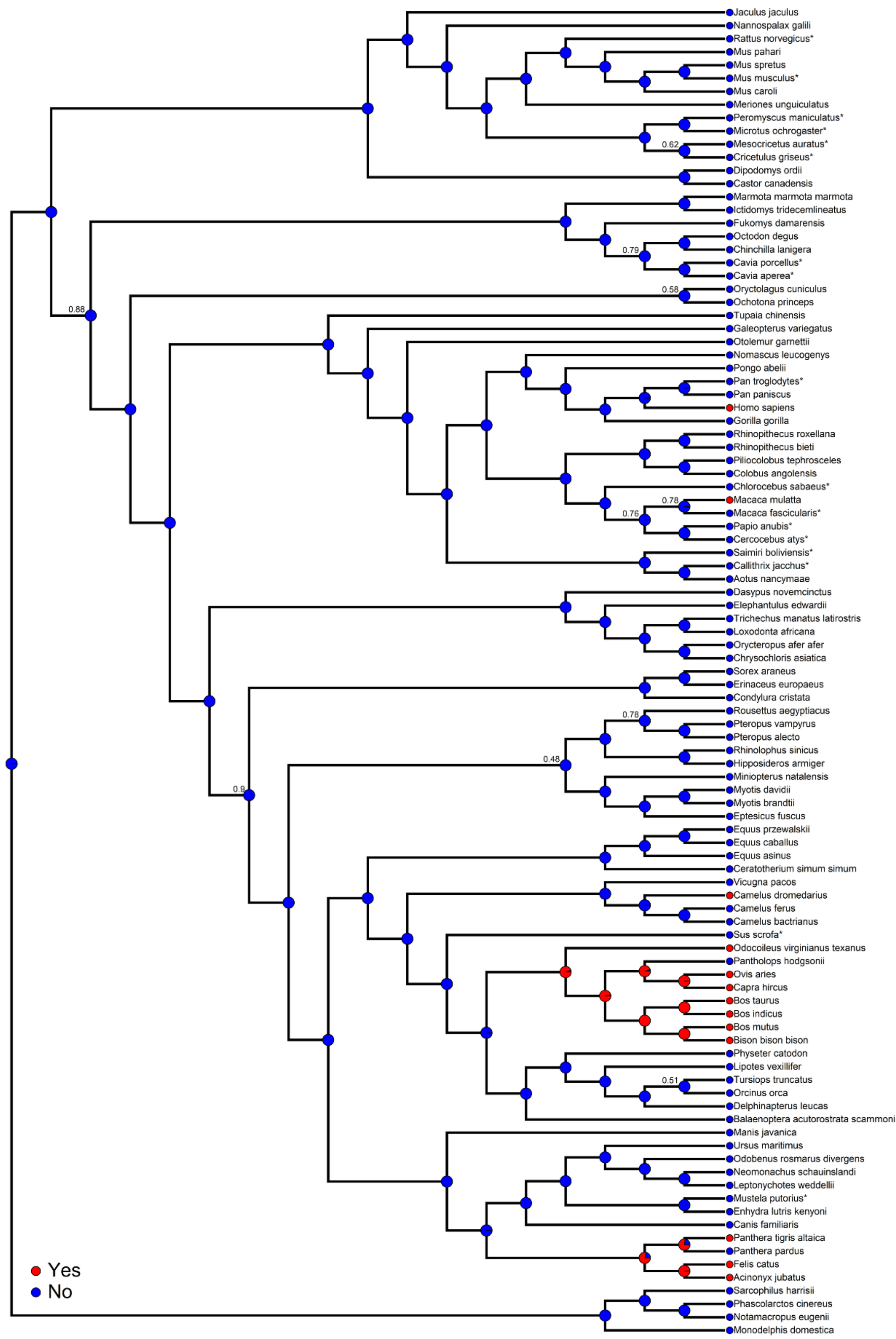


**Figure 3.4 Species Tree without Inoculated Species.** Species tree created using nucleotides and data excluding experimentally inoculated species.